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<p>(21) International Application Number: PCT/GB96/03221 (22) International Filing Date: 23 December 1996 (23.12.96) (30) Priority Data: 9526178.0 21 December 1995 (21.12.95) GB (71) Applicant (for all designated States except US): ST. GEORGE'S HOSPITAL MEDICAL SCHOOL [GB/GB]; Cranmer Terrace, London SW17 0RE (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): HERMON-TAYLOR, John [GB/GB]; St. George's Hospital Medical School, Dept. of Surgery, Cranmer Terrace, London SW17 0RE (GB). DO-RAN, Tim [AU/AU]; 1/8 Oxford Street, Whillington, VIC 3219 (AU). MILLAR, Douglas [GB/AU]; Csiro, Division of Biomolecular Engineering, P.O. Box 184, North Ryde, NSW 2113 (AU). TIZARD, Mark [GB/GB]; St. George's Hospital Medical School, Dept. of Surgery, Cranmer Terrace, London SW17 0RE (GB). LOUGHLIN, Mark [GB/GB]; St. George's Hospital Medical School, Dept. of Surgery, Cranmer Terrace, London SW17 0RE (GB). SUMAR, Nazira [GB/GB]; St. George's Hospital Medical School, Dept. of Surgery, Cranmer Terrace, London SW17 0RE (GB).</p>		<p>FORD, John [GB/GB]; St. George's Hospital Medical School, Dept. of Surgery, Cranmer Terrace, London SW17 0RE (GB). (74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp &amp; Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB). (81) Designated States: AU, CA, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i></p>
<p>(54) Title: NOVEL POLYNUCLEOTIDES AND POLYPEPTIDES IN PATHOGENIC MYCOBACTERIA AND THEIR USE AS DIAGNOSTICS, VACCINES AND TARGETS FOR CHEMOTHERAPY</p> <p>(57) Abstract</p> <p>The invention provides a nucleotide sequence representing a pathogenicity island found in species of pathogenic mycobacteria. The islands are shown as SEQ ID NOs: 3 and 4 and comprises several open reading frames encoding polypeptides. These polypeptides and their use in diagnosis and therapy form a further aspect of the invention.</p>		

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Novel polynucleotides and polypeptides in pathogenic mycobacteria and their use as diagnostics, vaccines and targets for chemotherapy.

5 This invention relates to the novel polynucleotide sequence we have designated "GS" which we have identified in pathogenic mycobacteria. GS is a pathogenicity island within 8kb of DNA comprising a core region of 5.75kb and an adjacent transmissible element within 2.25kb. GS is contained within *Mycobacterium paratuberculosis*, *Mycobacterium avium* subsp. *silvaticum* and some  
10 pathogenic isolates of *M. avium*. Functional portions of the core region of GS are also represented by regions with a high degree of homology that we have identified in cosmids containing genomic DNA from *Mycobacterium tuberculosis*.

15 Background to the invention

*Mycobacterium tuberculosis* (Mtb) is a major cause of global diseases of humans as well as animals. Although conventional methods of diagnosis including microscopy, culture and skin testing exist for the recognition of these diseases, improved  
20 methods particularly new immunodiagnostics and DNA-based detection systems are needed. Drugs used to treat tuberculosis are increasingly encountering the problem of resistant organisms. New drugs targeted at specific pathogenicity determinants as well as new vaccines for the prevention and treatment of tuberculosis  
25 are required. The importance of Mtb as a global pathogen is reflected in the commitment being made to sequencing the entire genome of this organism. This has generated a large amount of DNA sequence data of genomic DNA within cosmid and other libraries. Although the DNA sequence is known in the art, the  
30 functions of the vast majority of these sequences, the proteins they encode, the biological significance of these proteins, and the overall relevance and use of these genes and their products as diagnostics, vaccines and targets for chemotherapy for tuberculous disease, remains entirely unknown.

35 *Mycobacterium avium* subsp. *silvaticum* (Mavs) is a pathogenic mycobacterium causing diseases of animals and birds, but it can

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also affect humans. *Mycobacterium paratuberculosis* (Mptb) causes chronic inflammation of the intestine in many species of animals including primates and can also cause Crohn's disease in humans. Mptb is associated with other chronic inflammatory diseases of humans such as sarcoidosis. Subclinical Mptb infection is widespread in domestic livestock and is present in milk from infected animals. The organism is more resistant to pasteurisation than Mtb and can be conveyed to humans in retail milk supplies. Mptb is also present in water supplies, particularly those contaminated with run-off from heavily grazed pastures. Mptb and Mavs contain the insertion elements IS900 and IS902 respectively, and these are linked to pathogenicity in these organisms. IS900 and IS902 provide convenient highly specific multi-copy DNA targets for the sensitive detection of these organisms using DNA-based methods and for the diagnosis of infections in animals and humans. Much improvement is however required in the immunodiagnosis of Mptb and Mavs infections in animals and humans. Mptb and Mavs are in general, resistant in vivo to standard anti-tuberculous drugs. Although substantial clinical improvements in infections caused by Mptb, such as Crohn's disease, may result from treatment of patients with combinations of existing drugs such as Rifabutin, Clarithromycin or Azithromycin, additional effective drug treatments are required. Furthermore, there is an urgent need for effective vaccines for the prevention and treatment of Mptb and Mavs infections in animals and humans based upon the recognition of specific pathogenicity determinants.

Pathogenicity islands are, in general, 7-9kb regions of DNA comprising a core domain with multiple ORFs and an adjacent transmissible element. The transmissible element also encodes proteins which may be linked to pathogenicity, such as by providing receptors for cellular recognition. Pathogenicity islands are envisaged as mobile packages of DNA which, when they enter an organism, assist in bringing about its conversion from a non-disease-causing to a disease-causing strain.

#### Description of the Drawings

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Figure 1(a) and (b) shows a linear map of the pathogenicity island GS in *Mavs* (Fig 1a) and in *Mptb* (Fig 1b). The main open reading frames are illustrated as ORFs A to H. ORFs A to F are found within the core region of GS. ORFs G and H are encoded by the adjacent transmissible element portion of GS.

### Disclosure of the invention

Using a DNA-based differential analysis technology we have discovered and characterised a novel polynucleotide in *Mptb* (isolates 0022 from a Guernsey cow and 0021 from a red deer). This polynucleotide comprises the gene region we have designated GS. GS is found in *Mptb* using the identifier DNA sequences Seq.ID.No 1 and 2 where the Seq.ID No2 is the complementary sequence of Seq.ID No 1. GS is also identified in *Mavs*. The complete DNA sequence incorporating the positive strand of GS from an isolate of *Mavs* comprising 7995 nucleotides, including the core region of GS and adjacent transmissible element, is given in Seq.ID No.3. DNA sequence comprising 4435 bp of the positive strand of GS obtained from an isolate of *Mptb* including the core region of GS (nucleotides 1614 to 6047 of GS in *Mavs*) is given in Seq.ID No 4. The DNA sequence of GS from *Mptb* is highly (99.4%) homologous to GS in *Mavs*. The remaining portion of the DNA sequence of GS in *Mptb*, is readily obtainable by a person skilled in the art using standard laboratory procedures. The entire functional DNA sequence including core region and transmissible element of GS in *Mptb* and *Mavs* as described above, comprise the polynucleotide sequences of the invention.

There are 8 open reading frames (ORFs) in GS. Six of these designated GSA, GSB, GSC, GSD, GSE and GSF are encoded by the core DNA region of GS which, characteristically for a pathogenicity island, has a different GC content than the rest of the microbial genome. Two ORFs designated GSG and GSH are encoded by the transmissible element of GS whose GC content resembles that of the rest of the mycobacterial genome. The ORF GSH comprises two sub-ORFs H<sub>1</sub> H<sub>2</sub> on the complementary DNA strand linked by a programmed frameshifting site so that a single polypeptide is translated from the ORF GSH. The nucleotide

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sequences of the 8 ORFs in GS and their translations are shown in Seq. ID No 5 to Seq.ID No 29 as follows:

- 5      ORF A:      Seq. ID No 5 Nucleotides 50 to 427 of GS from *Mavs*  
                 Seq. ID No 6 Amino acid sequence encoded by Seq.ID No  
                 5.
- ORF B:      Seq. ID No 7 Nucleotides 772 to 1605 of GS from *Mavs*  
                 Seq. ID No 8 Amino acid sequence encoded by Seq.ID No  
                 7.
- 10      ORF C:      Seq. ID No 9 Nucleotides 1814 to 2845 of GS from *Mavs*  
                 Seq. ID No 10 Amino acid sequence encoded by Seq.ID No  
                 9.  
                 Seq. ID No 11 Nucleotides 201 to 1232 of GS from *Mptb*  
                 Seq. ID No 12 Amino acid sequence encoded by Seq.ID No  
                 11
- 15      ORF D:      Seq. ID No 13 Nucleotides 2785 to 3804 of GS from *Mavs*  
                 Seq. ID No 14 Amino acid sequence encoded by Seq.ID No  
                 13.  
                 Seq. ID No 15 Nucleotides 1172 to 2191 of GS from *Mptb*  
                 Seq. ID No 16 Amino acid sequence encoded by Seq.ID No  
20                   15.
- ORF E:      Seq. ID No 17 Nucleotides 4080 to 4802 of GS from *Mavs*  
                 Seq. ID No 18 Amino acid sequence encoded by Seq.ID No  
                 17.  
                 Seq. ID No 19 Nucleotides 2467 to 3189 of GS from *Mptb*  
25                   Seq. ID No 20 Amino acid sequence encoded by Seq.ID No  
                 19.
- ORF F:      Seq. ID No 21 Nucleotides 4947 to 5747 of GS from *Mavs*  
                 Seq. ID No 22 Amino acid sequence encoded by Seq.ID No  
                 21.  
30                   Seq. ID No 23 Nucleotides 3335 to 4135 of GS from *Mptb*  
                 Seq. ID No 24 Amino acid sequence encoded by Seq.ID No  
                 23.

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ORF G: Seq. ID No 25 Nucleotides 6176 to 7042 of GS from Mavs  
Seq. ID No 26 Amino acid sequence encoded by  
Seq.ID No 25.

ORF H: Seq.ID No 27 Nucleotides 7953 to 6215 from Mavs.

5 ORF H<sub>1</sub>: Seq.ID No 28 Amino acid sequence encoded by  
nucleotides 7953 to 7006 of Seq.ID No 27

ORF H<sub>2</sub>: Seq.ID No 29 Amino acid sequence encoded by  
nucleotides 7009 to 6215 of Seq.ID No 27

10 The polynucleotides in Mtb with homology to the ORFs B, C, E and  
F of GS in Mptb and Mavs, and the polypeptides they are now known  
to encode as a result of our invention, are as follows:

ORF B: Seq.ID No 30 Cosmid MTCY277 nucleotides 35493 to  
34705  
Seq.ID No 31 Amino acid sequence encoded by Seq.ID  
15 No30.

ORF C: Seq.ID No 32 Cosmid MTCY277 nucleotides 31972 to 32994  
Seq.ID No 33 Amino acid sequence encoded by Seq.ID  
No32.

20 ORF E: Seq.ID No 34 Cosmid MTCY277 nucleotides 34687 to 33956  
Seq.ID No 35 Amino acid sequence encoded by Seq.ID  
No34.

ORF E: Seq.ID No 36 Cosmid MTO24 nucleotides 15934 to 15203  
Seq.ID No 37 Amino acid sequence encoded by Seq.ID  
No36.

25 ORF F: Seq.ID No38 Cosmid MTO24 nucleotides 15133 to 14306  
Seq.ID No 39 Amino acid sequence encoded by Seq.ID  
No38.

The proteins and peptides encoded by the ORFs A to H in Mptb and  
Mavs and the amino acid sequences from homologous genes we have

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discovered in *Mtb* given in Seq.ID Nos 31, 33, 35, 37 and 39, as described above and fragments thereof, comprise the polypeptides of the invention. The polypeptides of the invention are believed to be associated with specific immunoreactivity and with the pathogenicity of the host micro-organisms from which they were obtained.

The present invention thus provides a polynucleotide in substantially isolated form which is capable of selectively hybridising to sequence ID Nos 3 or 4 or a fragment thereof. The polynucleotide fragment may alternatively comprise a sequence selected from the group of Seq.ID.No: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27. The invention further provides a polynucleotide in substantially isolated form whose sequence consists essentially of a sequence selected from the group Seq ID Nos. 30, 32, 34, 36 and 38, or a corresponding sequence selectively hybridizable thereto, or a fragment of said sequence or corresponding sequence.

The invention further provides diagnostic probes such as a probe which comprises a fragment of at least 15 nucleotides of a polynucleotide of the invention, or a peptide nucleic acid or similar synthetic sequence specific ligand, optionally carrying a revealing label. The invention also provides a vector carrying a polynucleotide as defined above, particularly an expression vector.

The invention further provides a polypeptide in substantially isolated form which comprises any one of the sequences selected from the group consisting Seq.ID.No: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 35, 37 and 39, or a polypeptide substantially homologous thereto. The invention additionally provides a polypeptide fragment which comprises a fragment of a polypeptide defined above, said fragment comprising at least 10 amino acids and an epitope. The invention also provides polynucleotides in substantially isolated form which encode polypeptides of the invention, and vectors which comprise such polynucleotides, as well as antibodies capable of binding such polypeptides. In an additional aspect, the invention provides

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kits comprising polynucleotides, polypeptides, antibodies or synthetic ligands of the invention and methods of using such kits in diagnosing the presence or absence of mycobacteria in a sample. The invention also provides pharmaceutical compositions comprising polynucleotides of the invention, polypeptides of the invention or antisense probes and the use of such compositions in the treatment or prevention of diseases caused by mycobacteria. The invention also provides polynucleotide prevention and treatment of infections due to GS-containing pathogenic mycobacteria in animals and humans and as a means of enhancing in vivo susceptibility of said mycobacteria to antimicrobial drugs. The invention also provides bacteria or viruses transformed with polynucleotides of the invention for use as vaccines. The invention further provides *Mptb* or *Mavs* in which all or part of the polynucleotides of the invention have been deleted or disabled to provide mutated organisms of lower pathogenicity for use as vaccines in animals and humans. The invention further provides *Mtb* in which all or part of the polynucleotides encoding polypeptides of the invention have been deleted or disabled to provide mutated organisms or lower pathogenicity for use as vaccines in animals and humans.

A further aspect of the invention is our discovery of homologies between the ORFs B, C and E in GS on the one hand, and *Mtb* cosmid MTCY277 on the other (data from Genbank database using the computer programmes BLAST and BLIXEM). The homologous ORFs in MTCY277 are adjacent to one another consistent with the form of another pathogenicity island in *Mtb*. A further aspect of the invention is our discovery of homologies between ORFs E and F in GS, and *Mtb* cosmid MTO24 (also Genbank, as above) with the homologous ORFs close to one another. The use of polynucleotides and polypeptides from *Mtb* (Seq. ID Nos 30, 31, 32, 33, 34, 35, 36, 37, 38 and 39) in substantially isolated form as diagnostics, vaccines and targets for chemotherapy, for the management and prevention of *Mtb* infections in humans and animals, and the processes involved in the preparation and use of these diagnostics, vaccines and new chemotherapeutic agents, comprise further aspects of the invention.

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Detailed description of the invention.A. Polynucleotides

Polynucleotides of the invention as defined herein may comprise DNA or RNA. They may also be polynucleotides which include within them synthetic or modified nucleotides or peptide nucleic acids. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to couple the said polynucleotide to a solid phase or to enhance the recognition, the in vivo activity, or the lifespan of polynucleotides of the invention.

A number of different types of polynucleotides of the invention are envisaged. In the broadest aspect, polynucleotides and fragments thereof capable of hybridizing to SEQ ID NO:3 or 4 form a first aspect of the invention. This includes the polynucleotide of SEQ ID NO: 3 or 4. Within this class of polynucleotides various sub-classes of polynucleotides are of particular interest.

One sub-class of polynucleotides which is of interest is the class of polynucleotides encoding the open reading frames A, B, C, D, E, F, G and H, including SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27. As discussed below, polynucleotides encoding ORF H include the polynucleotide sequences 7953 to 7006 and 7009 to 6215 within SEQ ID NO: 27, as well as modified sequences in which the frame-shift has been modified so that the two sub-reading frames are placed in a single reading frame. This may be desirable where the polypeptide is to be produced in recombinant expression systems.

The invention thus provides a polynucleotide in substantially isolated form which encodes any one of these ORFs or combinations

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thereof. Combinations thereof includes combinations of 2, 3, 4, 5 or all of the ORFs. Polynucleotides may be provided which comprise an individual ORF carried in a recombinant vector including the vectors described herein. Thus in one preferred  
5 aspect the invention provides a polynucleotide in substantially isolated form capable of selectively hybridizing to the nucleic acid comprising ORFs A to F of the core region of the *Mptb* and *Mavs* pathogenicity islands of the invention. Fragments thereof corresponding to ORFs A to E, B to F, A to D, B to E, A to C, B  
10 to D or any two adjacent ORFs are also included in the invention.

Polynucleotides of the invention will be capable of selectively hybridizing to the corresponding portion of the GS region, or to the corresponding ORFs of *Mtb* described herein. The term  
15 "selectively hybridizing" indicates that the polynucleotides will hybridize, under conditions of medium to high stringency (for example 0.03 M sodium chloride and 0.03 M sodium citrate at from about 50°C to about 60°C) to the corresponding portion of SEQ ID NO:3 or 4 or the complementary strands thereof but not to genomic DNA from mycobacteria which are usually non-pathogenic including  
20 non-pathogenic species of *M.avium*. Such polynucleotides will generally be generally at least 68%, e.g. at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the corresponding DNA of GS. The corresponding portion will be of over a region of at least 20, preferably at  
25 least 30, for instance at least 40, 60 or 100 or more contiguous nucleotides.

By "corresponding portion" it is meant a sequence from the GS region of the same or substantially similar size which has been determined, for example by computer alignment, to have the  
30 greatest degree of homology to the polynucleotide.

Any combination of the above mentioned degrees of homology and minimum sizes may be used to define polynucleotides of the invention, with the more stringent combinations (i.e. higher homology over longer lengths) being preferred. Thus for example  
35 a polynucleotide which is at least 80% homologous over 25, preferably 30 nucleotides forms one aspect of the invention, as

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does a polynucleotide which is at least 90% homologous over 40 nucleotides.

A further class of polynucleotides of the invention is the class of polynucleotides encoding polypeptides of the invention, the polypeptides of the invention being defined in section B below. Due to the redundancy of the genetic code as such, polynucleotides may be of a lower degree of homology than required for selective hybridization to the GS region. However, when such polynucleotides encode polypeptides of the invention these polynucleotides form a further aspect. It may for example be desirable where polypeptides of the invention are produced recombinantly to increase the GC content of such polynucleotides. This increase in GC content may result in higher levels of expression via codon usage more appropriate to the host cell in which recombinant expression is taking place.

An additional class of polynucleotides of the invention are those obtainable from cosmids MTCY277 and MT024 (containing *Mtb* genomic sequences), which polynucleotides consist essentially of the fragment of the cosmid containing an open reading frame encoding any one of the homologous ORFs B, C, E or F respectively. Such polynucleotides are referred to below as *Mtb* polynucleotides. However, where reference is made to polynucleotides in general such reference includes *Mtb* polynucleotides unless the context is explicitly to the contrary. In addition, the invention provides polynucleotides which encode the same polypeptide as the abovementioned ORFs of *Mtb* but which, due to the redundancy of the genetic code, have different nucleotide sequences. These form further *Mtb* polynucleotides of the invention. Fragments of *Mtb* polynucleotides suitable for use as probes or primers also form a further aspect of the invention.

The invention further provides polynucleotides in substantially isolated form capable of selectively hybridizing (where selectively hybridizing is as defined above) to the *Mtb* polynucleotides of the invention.

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The invention further provides the *Mtb* polynucleotides of the invention linked, at either the 5' and/or 3' end to polynucleotide sequences to which they are not naturally contiguous. Such sequences will typically be sequences found in cloning or expression vectors, such as promoters, 5' untranslated  
5 sequence, 3' untranslated sequence or termination sequences. The sequences may also include further coding sequences such as signal sequences used in recombinant production of proteins.

Further polynucleotides of the invention are illustrated in the  
10 accompanying examples.

Polynucleotides of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels  
15 or a probe linked covalently to a solid phase, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 or more nucleotides in length, and are also encompassed by the term polynucleotides  
20 of the invention as used herein.

Primers of the invention which are preferred include primers directed to any part of the ORFs defined herein. The ORFs from other isolates of pathogenic mycobacteria which contain a GS region may be determined and conserved regions within each  
25 individual ORF may be identified. Primers directed to such conserved regions form a further preferred aspect of the invention. In addition, the primers and other polynucleotides of the invention may be used to identify, obtain and isolate ORFs capable of selectively hybridizing to the polynucleotides of the  
30 invention which are present in pathogenic mycobacteria but which are not part of a pathogenicity island in that particular species of bacteria. Thus in addition to the ORFs B, C, E and F which have been identified in *Mtb*, similar ORFs may be identified in other pathogens and ORFs corresponding to the GS ORFs C, D, E,  
35 F and H, may also be identified.

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Polynucleotides such as DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

5 In general, primers will be produced by synthetic means, involving a step-wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art. Longer polynucleotides will generally be produced using  
10 recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair or primers (e.g. of about 15-30 nucleotides) to a region of GS, which it is desired to clone, bringing the primers into contact with genomic DNA from a mycobacterium or a vector carrying the  
15 GS sequence, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme  
20 recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

Such techniques may be used to obtain all or part of the GS or ORF sequences described herein, as well as further genomic clones containing full open reading frames. Although in general such  
25 techniques are well known in the art, reference may be made in particular to Sambrook J., Fritsch EF., Maniatis T (1989). Molecular cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory.

Polynucleotides which are not 100% homologous to the sequences  
30 of the present invention but fall within the scope of the invention can be obtained in a number of ways.

Other isolates or strains of pathogenic mycobacteria will be expected to contain allelic variants of the GS sequences described herein, and these may be obtained for example by  
35 probing genomic DNA libraries made from such isolates or strains

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of bacteria using GS or ORF sequences as probes under conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50°C to about 60°C).

- 5 A particularly preferred group of pathogenic mycobacteria are isolates of *M.paratuberculosis*. Polynucleotides based on GS regions from such bacteria are particularly preferred. Preferred fragments of such regions include fragments encoding individual open reading frames including the preferred groups and combinations of open reading frames discussed above.
- 10 Alternatively, such polynucleotides may be obtained by site directed mutagenesis of the GS or ORF sequences or allelic variants thereof. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the
- 15 polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides of the invention. Such altered property or function will include the addition of
- 20 amino acid sequences of consensus signal peptides known in the art to effect transport and secretion of the modified polypeptide of the invention. Another altered property will include metagenesis of a catalytic residue or generation of fusion proteins with another polypeptide. Such fusion proteins may be
- 25 with an enzyme, with an antibody or with a cytokine or other ligand for a receptor, to target a polypeptide of the invention to a specific cell type in vitro or in vivo.

The invention further provides double stranded polynucleotides comprising a polynucleotide of the invention and its complement.

- 30 Polynucleotides or primers of the invention may carry a revealing label. Suitable labels include radioisotopes such as <sup>32</sup>P or <sup>35</sup>S, enzyme labels, other protein labels or smaller labels such as biotin or fluorophores. Such labels may be added to polynucleotides or primers of the invention and may be detected
- 35 using by techniques known per se.

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Polynucleotides or primers of the invention or fragments thereof labelled or unlabelled may be used by a person skilled in the art in nucleic acid-based tests for the presence or absence of *Mptb*, *Mavs*, other GS-containing pathogenic mycobacteria, or *Mtb* applied to samples of body fluids, tissues, or excreta from animals and humans, as well as to food and environmental samples such as river or ground water and domestic water supplies.

Human and animal body fluids include sputum, blood, serum, plasma, saliva, milk, urine, csf, semen, faeces and infected discharges. Tissues include intestine, mouth ulcers, skin, lymph nodes, spleen, lung and liver obtained surgically or by a biopsy technique. Animals particularly include commercial livestock such as cattle, sheep, goats, deer, rabbits but wild animals and animals in zoos may also be tested.

Such tests comprise bringing a human or animal body fluid or tissue extract, or an extract of an environmental or food sample, into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and detecting any duplex formed between the probe and nucleic acid in the sample. Such detection may be achieved using techniques such as PCR or by immobilising the probe on a solid support, removing nucleic acid in the sample which is not hybridized to the probe, and then detecting nucleic acid which has hybridized to the probe. Alternatively, the sample nucleic acid may be immobilized on a solid support, and the amount of probe bound to such a support can be detected. Suitable assay methods of this any other formats can be found in for example WO89/03891 and WO90/13667.

Polynucleotides of the invention or fragments thereof labelled or unlabelled may also be used to identify and characterise different strains of *Mptb*, *Mavs*, other GS-containing pathogenic mycobacteria, or *Mtb*, and properties such as drug resistance or susceptibility.

The probes of the invention may conveniently be packaged in the form of a test kit in a suitable container. In such kits the probe may be bound to a solid support where the assay format for

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which the kit is designed requires such binding. The kit may also contain suitable reagents for treating the sample to be probed, hybridising the probe to nucleic acid in the sample, control reagents, instructions, and the like.

- 5 The use of polynucleotides of the invention in the diagnosis of inflammatory diseases such as Crohn's disease or sarcoidosis in humans or Johne's disease in animals form a preferred aspect of the invention. The polynucleotides may also be used in the prognosis of these diseases. For example, the response of a  
10 human or animal subject in response to antibiotic, vaccination or other therapies may be monitored by utilizing the diagnostic methods of the invention over the course of a period of treatment and following such treatment.

- The use of *Mtb* polynucleotides (particularly in the form of  
15 probes and primers) of the invention in the above-described methods form a further aspect of the invention, particularly for the detection, diagnosis or prognosis of *Mtb* infections.

#### B. Polypeptides.

- Polypeptides of the invention include polypeptides in  
20 substantially isolated form encoded by GS. This includes the full length polypeptides encoded by the positive and complementary negative strands of GS. Each of the full length polypeptides will contain one of the amino acid sequences set out in Seq ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and  
25 29. Polypeptides of the invention further include variants of such sequences, including naturally occurring allelic variants and synthetic variants which are substantially homologous to said polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, e.g. 80%, 90%, 95% or 98%  
30 amino acid homology (identity) over 30 or more, e.g. 40, 50 or 100 amino acids. For example, one group of substantially homologous polypeptides are those which have at least 95% amino acid identity to a polypeptide of any one of Seq ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 29 over their entire length.  
35 Even more preferably, this homology is 98%.

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Polypeptides of the invention further include the polypeptide sequences of the homologous ORFs of *Mtb*, namely Seq ID Nos. 31, 33, 35, 37 and 39. Unless explicitly specified to the contrary, reference to polypeptides of the invention and their fragments include these *Mtb* polypeptides and fragments, and variants thereof (substantially homologous to said sequences) as defined herein.

Polypeptides of the invention may be obtained by the standard techniques mentioned above. Polypeptides of the invention also include fragments of the above mentioned full length polypeptides and variants thereof, including fragments of the sequences set out in SEQ ID NOS:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 35, 37 and 39. Such fragments for example of 8, 10, 12, 15 or up to 30 or 40 amino acids may also be obtained synthetically using standard techniques known in the art.

Preferred fragments include those which include an epitope, especially an epitope which is specific to the pathogenicity of the mycobacterial cell from which the polypeptide is derived. Suitable fragments will be at least about 5, e.g. 8, 10, 12, 15 or 20 amino acids in size, or larger. Epitopes may be determined either by techniques such as peptide scanning techniques as described by Geysen et al, *Mol.Immunol.*, 23; 709-715 (1986), as well as other techniques known in the art.

The term "an epitope which is specific to the pathogenicity of the mycobacterial cell" means that the epitope is encoded by a portion of the GS region, or by the corresponding ORF sequences of *Mtb* which can be used to distinguish mycobacteria which are pathogenic by from related non-pathogenic mycobacteria including non-pathogenic species of *M.avium*. This may be determined using routine methodology. A candidate epitope from an ORF may be prepared and used to immunise an animal such as a rat or rabbit in order to generate antibodies. The antibodies may then be used to detect the presence of the epitope in pathogenic mycobacteria and to confirm that non-pathogenic mycobacteria do not contain any proteins which react with the epitope. Epitopes may be linear or conformational.

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Polypeptides of the invention may be in a substantially isolated form. It will be understood that the polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and still be regarded as substantially isolated. A polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is a polypeptide of the invention.

- 10 Polypeptides of the invention may be modified to confer a desired property or function for example by the addition of Histidine residues to assist their purification or by the addition of a signal sequence to promote their secretion from a cell.

Thus, polypeptides of the invention include fusion proteins which  
15 comprise a polypeptide encoding all or part of one or more of an ORF of the invention fused at the N- or C-terminus to a second sequence to provide the desired property or function. Sequences which promote secretion from a cell include, for example the yeast  $\alpha$ -factor signal sequence.

- 20 A polypeptide of the invention may be labelled with a revealing label. The revealing label may be any suitable label which allows the polypeptide to be detected. Suitable labels include radioisotopes, e.g.  $^{125}\text{I}$ ,  $^{35}\text{S}$  enzymes, antibodies, polynucleotides and ligands such as biotin. Labelled polypeptides of the  
25 invention may be used in diagnostic procedures such as immunoassays in order to determine the amount of a polypeptide of the invention in a sample. Polypeptides or labelled polypeptides of the invention may also be used in serological or cell mediated immune assays for the detection of immune  
30 reactivity to said polypeptides in animals and humans using standard protocols.

A polypeptide or labelled polypeptide of the invention or fragment thereof may also be fixed to a solid phase, for example the surface of an immunoassay well, microparticle, dipstick or  
35 biosensor. Such labelled and/or immobilized polypeptides may be

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packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

Such polypeptides and kits may be used in methods of detection of antibodies or cell mediated immunoreactivity, to the mycobacterial proteins and peptides encoded by the ORFs of the invention and their allelic variants and fragments, using immunoassay. Such host antibodies or cell mediated immune reactivity will occur in humans or animals with an immune system which detects and reacts against polypeptides of the invention. The antibodies may be present in a biological sample from such humans or animals, where the biological sample may be a sample as defined above particularly blood, milk or saliva.

Immunoassay methods are well known in the art and will generally comprise:

- (a) providing a polypeptide of the invention comprising an epitope bindable by an antibody against said mycobacterial polypeptide;
- (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and
- (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

Immunoassay methods for cell mediated immune reactivity in animals and humans are also well known in the art (e.g. as described by Weir et al 1994, J.Immunol Methods 176; 93-101) and will generally comprise

- (a) providing a polypeptide of the invention comprising an epitope bindable by a lymphocyte or macrophage or other cell receptor;
- (b) incubating a cell sample with said polypeptide under conditions which allow for a cellular immune response such as release of cytokines or other mediator to occur; and
- (c) detecting the presence of said cytokine or mediator in the incubate.

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Polypeptides of the invention may be made by standard synthetic means well known in the art or recombinantly, as described below.

Polypeptides of the invention or fragments thereof labelled or unlabelled may also be used to identify and characterise  
5 different strains of *Mptb*, *Mavs*, other GS-containing pathogenic mycobacteria, or *Mtb*, and properties such as drug resistance or susceptibility.

The polypeptides of the invention may conveniently be packaged in the form of a test kit in a suitable container. In such kits  
10 the polypeptide may be bound to a solid support where the assay format for which the kit is designed requires such binding. The kit may also contain suitable reagents for treating the sample to be examined, control reagents, instructions, and the like.

The use of polypeptides of the invention in the diagnosis of  
15 inflammatory diseases such as Crohn's disease or sarcoidosis in humans or Johne's disease in animals form a preferred aspect of the invention. The polypeptides may also be used in the prognosis of these diseases. For example, the response of a human or animal subject in response to antibiotic or other  
20 therapies may be monitored by utilizing the diagnostic methods of the invention over the course of a period of treatment and following such treatment.

The use of *Mtb* polypeptides of the invention in the above-described methods form a further aspect of the invention,  
25 particularly for the detection, diagnosis or prognosis of *Mtb* infections.

Polypeptides of the invention may also be used in assay methods for identifying candidate chemical compounds which will be useful in inhibiting, binding to or disrupting the function of said  
30 polypeptides required for pathogenicity. In general, such assays involve bringing the polypeptide into contact with a candidate inhibitor compound and observing the ability of the compound to disrupt, bind to or interfere with the polypeptide.

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There are a number of ways in which the assay may be formatted. For example, those polypeptides which have an enzymatic function may be assayed using labelled substrates for the enzyme, and the amount of, or rate of, conversion of the substrate into a product measured, e.g by chromatography such as HPLC or by a colourimetric assay. Suitable labels include  $^{35}\text{S}$ ,  $^{125}\text{I}$ , biotin or enzymes such as horse radish peroxidase.

For example, the gene product of ORF C is believed to have GDP-mannose dehydratase activity. Thus an assay for inhibitors of the gene product may utilise for example labelled GDP-mannose, GDP or mannose and the activity of the gene product followed. ORF D encodes a gene related to the synthesis and regulation of capsular polysaccharides, which are often associated with invasiveness and pathogenicity. Labelled polysaccharide substrates may be used in assays of the ORF D gene product. The gene product of ORF F encodes a protein with putative glucosyl transferase activity and thus labelled amino sugars such as  $\beta$ -1-3-N-acetylglucosamine may be used as substrates in assays.

Candidate chemical compounds which may be used may be natural or synthetic chemical compounds used in drug screening programmes. Extracts of plants which contain several characterised or uncharacterised components may also be used.

Alternatively, the a polypeptide of the invention may be screened against a panel of peptides, nucleic acids or other chemical functionalities which are generated by combinatorial chemistry. This will allow the definition of chemical entities which bind to polypeptides of the invention. Typically, the polypeptide of the invention will be brought into contact with a panel of compounds from a combinatorial library, with either the panel or the polypeptide being immobilized on a solid phase, under conditions suitable for the polypeptide to bind to the panel. The solid phase will then be washed under conditions in which only specific interactions between the polypeptide and individual members of the panel are retained, and those specific members may be utilized in further assays or used to design further panels of candidate compounds.

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For example, a number of assay methods to define peptide interaction with peptides are known. For example, WO86/00991 describes a method for determining mimotopes which comprises making panels of catamer preparations, for example octamers of amino acids, at which one or more of the positions is defined and the remaining positions are randomly made up of other amino acids, determining which catamer binds to a protein of interest and re-screening the protein of interest against a further panel based on the most reactive catamer in which one or more additional designated positions are systematically varied. This may be repeated throughout a number of cycles and used to build up a sequence of a binding candidate compound of interest.

WO89/03430 describes screening methods which permit the preparation of specific mimotopes which mimic the immunological activity of a desired analyte. These mimotopes are identified by reacting a panel of individual peptides wherein said peptides are of systematically varying hydrophobicity, amphipathic characteristics and charge patterns, using an antibody against an antigen of interest. Thus in the present case antibodies against the a polypeptide of the inventoin may be employed and mimotope peptides from such panels may be identified.

#### C. Vectors.

Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells are described below in connection with expression vectors.

#### D. Expression Vectors.

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Preferably, a polynucleotide of the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably  
5 linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the  
10 control sequences. Such vectors may be transformed into a suitable host cell as described above to provide for expression of a polypeptide of the invention. Thus, in a further aspect the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell  
15 transformed or transfected with an expression vector as described above, under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

A further embodiment of the invention provides vectors for the  
20 replication and expression of polynucleotides of the invention, or fragments thereof. The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The  
25 vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector  
30 may also be adapted to be used *in vivo*, for example in a method of naked DNA vaccination or gene therapy. A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of polynucleotides of the invention, including the DNA of GS, the  
35 open reading frames thereof and other corresponding ORFs particularly ORFs B, C, E and F from Mtb. The cells will be chosen to be compatible with the said vector and may for example be bacterial, yeast, insect or mammalian.

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Expression vectors are widely available in the art and can be obtained commercially. Mammalian expression vectors may comprise a mammalian or viral promoter. Mammalian promoters include the metallothionien promoter. Viral promoters include promoters from  
5 adenovirus, the SV40 large T promoter and retroviral LTR promoters. Promoters compatible with insect cells include the polyhedrin promoter. Yeast promoters include the alcohol dehydrogenase promoter. Bacterial promoters include the  $\beta$ -galactosidase promoter.

10 The expression vectors may also comprise enhancers, and in the case of eukaryotic vectors polyadenylation signal sequence downstream of the coding sequence being expressed.

Polypeptides of the invention may be expressed in suitable host cells, for example bacterial, yeast, plant, insect and mammalian  
15 cells, and recovered using standard purification techniques including, for example affinity chromatography, HPLC or other chromatographic separation techniques.

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation in  
20 order to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides or ligands may also be produced by synthetic means. Such antisense polynucleotides may be used in a method of controlling the levels of the proteins encoded by the ORFs of the invention in a mycobacterial cell.

25 Polynucleotides of the invention may also be carried by vectors suitable for gene therapy methods. Such gene therapy methods include those designed to provide vaccination against diseases caused by pathogenic mycobacteria or to boost the immune response of a human or animal infected with a pathogenic mycobacteria.

30 For example, Ziegner et al, AIDS, 1995, 9;43-50 describes the use of a replication defective recombinant amphotropic retrovirus to boost the immune response in patients with HIV infection. Such a retrovirus may be modified to carry a polynucleotide encoding a polypeptide or fragment thereof of the invention and the

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retrovirus delivered to the cells of a human or animal subject in order to provide an immune response against said polypeptide. The retrovirus may be delivered directly to the patient or may be used to infect cells ex-vivo, e.g. fibroblast cells, which are then introduced into the patient, optionally after being inactivated. The cells are desirably autologous or HLA-matched cells from the human or animal subject.

Gene therapy methods including methods for boosting an immune response to a particular pathogen are disclosed generally in for example WO95/14091, the disclosure of which is incorporated herein by reference. Recombinant viral vectors include retroviral vectors, adenoviral vectors, adeno-associated viral vectors, vaccinia virus vectors, herpes virus vectors and alphavirus vectors. Alpha virus vectors are described in, for example, WO95/07994, the disclosure of which is incorporated herein by reference.

Where direct administration of the recombinant viral vector is contemplated, either in the form of naked nucleic acid or in the form of packaged particles carrying the nucleic acid this may be done by any suitable means, for example oral administration or intravenous injection. From  $10^5$  to  $10^8$  c.f.u of virus represents a typical dose, which may be repeated for example weekly over a period of a few months. Administration of autologous or HLA-matched cells infected with the virus may be more convenient in some cases. This will generally be achieved by administering doses, for example from  $10^5$  to  $10^8$  cells per dose which may be repeated as described above.

The recombinant viral vector may further comprise nucleic acid capable of expressing an accessory molecule of the immune system designed to increase the immune response. Such a molecule may be for example interferon, particularly interferon gamma, an interleukin, for example IL- $1\alpha$ , IL- $1\beta$  or IL-2, or an HLA class I or II molecule. This may be particularly desirable where the vector is intended for use in the treatment of humans or animals already infected with a mycobacteria and it is desired to boost the immune response.

E. Antibodies.

The invention also provides monoclonal or polyclonal antibodies to polypeptides of the invention or fragments thereof. The invention further provides a process for the production of  
5 monoclonal or polyclonal antibodies to polypeptides of the invention. Monoclonal antibodies may be prepared by conventional hybridoma technology using the polypeptides of the invention or peptide fragments thereof, as immunogens. Polyclonal antibodies  
10 may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a polypeptide of the invention or peptide fragment thereof and recovering immune serum.

In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof  
15 haptenised to another polypeptide for use as immunogens in animals or humans.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments of whole antibodies which retain their binding activity for a polypeptide of the  
20 invention. Such fragments include Fv, F(ab') and F(ab')<sub>2</sub> fragments, as well as single chain antibodies. Furthermore, the antibodies and fragments thereof may be humanised antibodies, e.g. as described in EP-A-239400.

Antibodies may be used in methods of detecting polypeptides of  
25 the invention present in biological samples (where such samples include the human or animal body samples, and environmental samples, mentioned above) by a method which comprises:

- (a) providing an antibody of the invention;
- (b) incubating a biological sample with said antibody  
30 under conditions which allow for the formation of an antibody-antigen complex; and
- (c) determining whether antibody-antigen complex comprising said antibody is formed.

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Antibodies of the invention may be bound to a solid support for example an immunoassay well, microparticle, dipstick or biosensor and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

- 5 Antibodies of the invention may be used in the detection, diagnosis and prognosis of diseases as described above in relation to polypeptides of the invention.

#### F. Compositions.

- 10 The present invention also provides compositions comprising a polynucleotide or polypeptide of the invention together with a carrier or diluent. Compositions of the invention also include compositions comprising a nucleic acid, particularly and expression vector, of the invention. Compositions further include those carrying a recombinant virus of the invention.
- 15 Such compositions include pharmaceutical compositions in which case the carrier or diluent will be pharmaceutically acceptable.

- Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for inhalation as well as oral, parenteral (e.g. intramuscular or intravenous or transcutaneous)
- 20 administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In
- 25 general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

- For example, formulations suitable for parenteral administration
- 30 include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening

agents, and liposomes or other microparticulate systems which are designed to target the polynucleotide or the polypeptide of the invention to blood components or one or more organs, or to target cells such as M cells of the intestine after oral administration.

5 G. Vaccines.

In another aspect, the invention provides novel vaccines for the prevention and treatment of infections caused by *Mptb*, *Mavs*, other GS-containing pathogenic mycobacteria and *Mtb* in animals and humans. The term "vaccine" as used herein means an agent  
10 used to stimulate the immune system of a vertebrate, particularly a warm blooded vertebrate including humans, so as to provide protection against future harm by an organism to which the vaccine is directed or to assist in the eradication of an organism in the treatment of established infection. The immune  
15 system will be stimulated by the production of cellular immunity antibodies, desirably neutralizing antibodies, directed to epitopes found on or in a pathogenic mycobacterium which expresses any one of the ORFs of the invention. The antibody so produced may be any of the immunological classes, such as the  
20 immunoglobulins A, D, E, G or M. Vaccines which stimulate the production of IgA are interest since this is the principle immunoglobulin produced by the secretory system of warm-blooded animals, and the production of such antibodies will help prevent infection or colonization of the intestinal tract. However an  
25 IgM and IgG response will also be desirable for systemic infections such as Crohn's disease or tuberculosis.

Vaccines of the invention include polynucleotides of the invention or fragments thereof in suitable vectors and administered by injection of naked DNA using standard protocols.  
30 Polynucleotides of the invention or fragments thereof in suitable vectors for the expression of the polypeptides of the invention may be given by injection, inhalation or by mouth. Suitable vectors include *M.bovis* BCG, *M.smegmatis* or other mycobacteria, *Corynebacteria*, *Salmonella* or other agents according to  
35 established protocols.

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Polypeptides of the invention or fragments thereof in substantially isolated form may be used as vaccines by injection, inhalation, oral administration or by transcutaneous application according to standard protocols. Adjuvants (such as Iscoms or  
5 polylactide-coglycolide encapsulation), cytokines such as IL-12 and other immunomodulators may be used for the selective enhancement of the cell mediated or humoral immunological responses. Vaccination with polynucleotides and/or polypeptides  
10 of the invention may be undertaken to increase the susceptibility of pathogenic mycobacteria to antimicrobial agents *in vivo*.

In instances wherein the polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier.

A number of techniques for obtaining such linkage are known in  
15 the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridylthio) propionate (SPDP) and succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Illinois, (if the peptide lacks  
20 a sulfhydryl group, this can be provided by addition of a cysteine residue). These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino on a lysine, or  
other free amino group in the other. A variety of such disulfide/amide-forming agents are known. See, for example,  
25 Immun Rev (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thioether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic  
30 acid, and the like. The carboxyl group can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in EPO  
35 Pub. No. 259,149, the disclosure of which is incorporated herein by reference. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

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Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized  
5 Sepharose®, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, polylactide-coglycolide and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin  
10 molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

The immunogenicity of the epitopes may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example,  
15 that associated with hepatitis B surface antigen. See, e.g., US-A-4,722,840. Constructs wherein the epitope is linked directly to the particle-forming protein coding sequences produce hybrids which are immunogenic with respect to the epitope. In addition, all of the vectors prepared include epitopes specific to HBV,  
20 having various degrees of immunogenicity, such as, for example, the pre-S peptide.

In addition, portions of the particle-forming protein coding sequence may be replaced with codons encoding an epitope of the invention. In this replacement, regions which are not required  
25 to mediate the aggregation of the units to form immunogenic particles in yeast or mammals can be deleted, thus eliminating additional HBV antigenic sites from competition with the epitope of the invention.

Vaccines may be prepared from one or more immunogenic  
30 polypeptides of the invention. These polypeptides may be expressed in various host cells (e.g., bacteria, yeast, insect, or mammalian cells), or alternatively may be isolated from viral preparations or made synthetically.

In addition to the above, it is also possible to prepare live  
35 vaccines of attenuated microorganisms which express one or more

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recombinant polypeptides of the invention. Suitable attenuated microorganisms are known in the art and include, for example, viruses (e.g., vaccinia virus), as well as bacteria.

5 The preparation of vaccines which contain an immunogenic polypeptide(s) as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, or as suitably encapsulated oral preparations and either liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to ingestion or injection may also  
10 be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline,  
15 dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may  
20 be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine  
25 (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween® 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies  
30 directed against an immunogenic polypeptide containing an antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

The vaccines are conventionally administered parenterally, by  
35 injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories, oral formulations or as

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enemas. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% - 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% - 95% of active ingredient, preferably 25% - 70%.

The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 $\mu$ g to 250 $\mu$ g, of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, mode of administration and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgement of the practitioner and may be peculiar to each subject.

The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals

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required to maintain and or reenforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the  
5 individual and be dependent upon the judgement of the practitioner.

In a further aspect of the invention, there is provided an attenuated vaccine comprising a normally pathogenic mycobacteria which harbours an attenuating mutation in any one of the genes  
10 encoding a polypeptide of the invention. The gene is selected from the group of ORFs A, B, C, D, E, F, G and H, including the homologous ORFs B, C, E and F in *Mtb*.

The mycobacteria may be used in the form of killed bacteria or as a live attenuated vaccine. There are advantages to a live  
15 attenuated vaccine. The whole live organism is used, rather than dead cells or selected cell components which may exhibit modified or denatured antigens. Protein antigens in the outer membrane will maintain their tertiary and quaternary structures. Therefore the potential to elicit a good protective long term  
20 immunity should be higher.

The term "mutation" and the like refers to a genetic lesion in a gene which renders the gene non-functional. This may be at either the level of transcription or translation. The term thus envisages deletion of the entire gene or substantial portions  
25 thereof, and also point mutations in the coding sequence which result in truncated gene products unable to carry out the normal function of the gene.

A mutation introduced into a bacterium of the invention will generally be a non-reverting attenuating mutation. Non-reverting  
30 means that for practical purposes the probability of the mutated gene being restored to its normal function is small, for example less than 1 in  $10^6$  such as less than 1 in  $10^9$  or even less than 1 in  $10^{12}$ .

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An attenuated mycobacteria of the invention may be in isolated form. This is usually desirable when the bacterium is to be used for the purposes of vaccination. The term "isolated" means that the bacterium is in a form in which it can be cultured, processed or otherwise used in a form in which it can be readily identified and in which it is substantially uncontaminated by other bacterial strains, for example non-attenuated parent strains or unrelated bacterial strains. The term "isolated bacterium" thus encompasses cultures of a bacterial mutant of the invention, for example in the form of colonies on a solid medium or in the form of a liquid culture, as well as frozen or dried preparations of the strains.

In a preferred aspect, the attenuated mycobacterium further comprises at least one additional mutation. This may be a mutation in a gene responsible for the production of products essential to bacterial growth which are absent in a human or animal host. For example, mutations to the gene for aspartate semi-aldehyde dehydrogenase (*asd*) have been proposed for the production of attenuated strains of *Salmonella*. The *asd* gene is described further in Gene (1993) 129; 123-128. A lesion in the *asd* gene, encoding the enzyme aspartate  $\beta$ -semialdehyde dehydrogenase would render the organism auxotrophic for the essential nutrient diaminopellic acid (DAP), which can be provided exogenously during bulk culture of the vaccine strain. Since this compound is an essential constituent of the cell wall for gram-negative and some gram-positive organisms and is absent from mammalian or other vertebrate tissues, mutants would undergo lysis after about three rounds of division in such tissues. Analogous mutations may be made to the attenuated mycobacteria of the invention.

In addition or in the alternative, the attenuated mycobacteria may carry a *recA* mutation. The *recA* mutation knocks out homologous recombination - the process which is exploited for the construction of the mutations. Once the *recA* mutation has been incorporated the strain will be unable to repair the constructed deletion mutations. Such a mutation will provide attenuated strains in which the possibility of homologous recombination to

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with DNA from wild-type strains has been minimized. RecA genes have been widely studied in the art and their sequences are available. Further modifications may be made for additional safety.

- 5 The invention further provides a process for preparing a vaccine composition comprising an attenuated bacterium according to the invention process comprises (a) inoculating a culture vessel containing a nutrient medium suitable for growth of said bacterium; (b) culturing said bacterium; (c) recovering said  
10 bacteria and (d) mixing said bacteria with a pharmaceutically acceptable diluent or carrier.

Attenuated bacterial strains according to the invention may be constructed using recombinant DNA methodology which is known per se. In general, bacterial genes may be mutated by a process of  
15 targeted homologous recombination in which a DNA construct containing a mutated form of the gene is introduced into a host bacterium which it is desired to attenuate. The construct will recombine with the wild-type gene carried by the host and thus the mutated gene may be incorporated into the host genome to  
20 provide a bacterium of the present invention which may then be isolated.

The mutated gene may be obtained by introducing deletions into the gene, e.g by digesting with a restriction enzyme which cuts the coding sequence twice to excise a portion of the gene and  
25 then religating under conditions in which the excised portion is not reintroduced into the cut gene. Alternatively frame shift mutations may be introduced by cutting with a restriction enzyme which leaves overhanging 5' and 3' termini, filling in and/or trimming back the overhangs, and religating. Similar mutations  
30 may be made by site directed mutagenesis. These are only examples of the types of techniques which will readily be at the disposal of those of skill in the art.

Various assays are available to detect successful recombination. In the case of attenuations which mutate a target gene necessary  
35 for the production of an essential metabolite or catabolite

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compound, selection may be carried out by screening for bacteria unable to grow in the absence of such a compound. Bacteria may also be screened with antibodies or nucleic acids of the invention to determine the absence of production of a mutated  
5 gene product of the invention or to confirm that the genetic lesion introduced - e.g. a deletion - has been incorporated into the genome of the attenuated strain.

The concentration of the attenuated strain in the vaccine will be formulated to allow convenient unit dosage forms to be  
10 prepared. Concentrations of from about  $10^4$  to  $10^9$  bacteria per ml will generally be suitable, e.g. from about  $10^5$  to  $10^8$  such as about  $10^6$  per ml. Live attenuated organisms may be administered subcutaneously or intramuscularly at up to  $10^8$  organisms in one or more doses, e.g. from around  $10^5$  to  $10^8$ , e.g. about  $10^6$  or  $10^7$   
15 organisms in a single dose.

The vaccines of the invention may be administered to recipients to treat established disease or in order to protect them against diseases caused by the corresponding wild type mycobacteria, such as inflammatory diseases such as Crohn's disease or sarcoidosis  
20 in humans or Johne's disease in animals. The vaccine may be administered by any suitable route. In general, subcutaneous or intramuscular injection is most convenient, but oral, intranasal and colorectal administration may also be used.

The following Examples illustrates aspects of the invention.

#### 25 EXAMPLE 1

Tests for the presence of the GS identifier sequence were performed on  $5\mu\text{l}$  bacterial DNA extracts ( $25\mu\text{g/ml}$  to  $500\mu\text{g/ml}$ ) using polymerase chain reaction based on the oligonucleotide primers 5'-GATGCCGTGAGGAGGTAAAGCTGC-3' (Seq ID No. 40) and 5'-  
30 GATACGGCTCTTGAATCCTGCACG-3' (Seq ID No. 41) from within the identifier DNA sequences (Seq.ID Nos 1 and 2). PCR was performed for 40 cycles in the presence of 1.5 mM magnesium and an annealing temperature of  $58^\circ\text{C}$ . The presence or absence of the correct amplification product indicated the presence or absence

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of GS identifier sequence in the corresponding bacterium. GS identifier sequence is shown to be present in all the laboratory and field strains of *Mptb* and *Mavs* tested. This includes *Mptb* isolates 0025 (bovine CVL Weybridge), 0021 (caprine, Moredun), 5 0022 (bovine, Moredun), 0139 (human, Chiodini 1984), 0209, 0208, 0211, 0210, 0212, 0207, 0204, 0206 (bovine, Whipple 1990). All *Mptb* strains were IS900 positive. The *Mavs* strains include 0010 and 0012 (woodpigeon, Thorel) 0018 (armadillo, Portael) and 0034, 0037, 0038, 0040 (AIDS, Hoffner). All *Mavs* strains were 10 IS902 positive. One pathogenic *M.avium* strain 0033 (AIDS, Hoffner) also contained GS identifier sequence. GS identifier sequence is absent from other mycobacteria including other *M.avium*, *M.malmoense*, *M.szulgai*, *M.gordonae*, *M.chelonae*, *M.fortuitum*, *M.phlei*, as well as *E.coli*, *S.areus*, *Nocardia* sp, 15 *Streptococcus* sp. *Shigella* sp. *Pseudomonas* sp.

#### Example 2:

To obtain the full sequence of GS in *Mavs* and *Mptb* we generated a genomic library of *Mavs* using the restriction endonuclease EcoRI and cloning into the vector pUC18. This achieved a 20 representative library which was screened with <sup>32</sup>P-labelled identifier sequence yielding a positive clone containing a 17kbp insert. We constructed a restriction map of this insert and identified GS as fragments unique to *Mavs* and *Mptb* and not occurring in laboratory strains of *M.avium*. These fragments 25 were sub-cloned into pUC18 and pGEM4Z. We identified GS contained within an 8kb region. The full nucleotide sequence was determined for GS on both DNA strands using primer walking and automated DNA sequencing. DNA sequence for GS in *Mptb* was obtained using overlapping PCR products generated using PwoDNA 30 polymerase, a proofreading thermostable enzyme. The final DNA sequences were derived using the University of Wisconsin GCG gel assembly software package.

#### Example 3:

The DNA sequence of GS in *Mavs* and *Mptb* was found to be more 35 than 99% homologous. The ORFs encoded in GS were identified using GeneRunner and DNASTar computer programmes. Eight ORFs were identified and designated GSA, GSB, GSC, GSD, GSE, GSF, GSG

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and GSH. Database comparisons were carried out against the GenEMBL Database release version 48.0 (9/96), using the BLAST and BLIXEM programmes. GSA and GSB encoded proteins of 13.5kDa and 30.7kDa respectively, both of unknown functions. GSC encoded  
5 a protein of 38.4kDa with a 65% homology to the amino acid sequence of *rfbD* of *V.cholerae*, a 62% amino acid sequence homology to *gmd* of *E.coli* and a 58% homology to *gca* of *Ps.aeruginosa* which are all GDP-D-mannose dehydratases. Equivalent gene products in *H.influenzae*, *S.dysenteriae*,  
10 *Y.enterocolitica*, *N.gonorrhoea*, *K.pneumoniae* and *rfbD* in *Salmonella enterica* are all involved in 'O'-antigen processing known to be linked to pathogenicity. GSD encoded a protein of 37.1kDa which showed 58% homology at the DNA level to *wcaG* from *E.coli*, a gene involved in the synthesis and regulation of  
15 capsular polysaccharides, also related to pathogenicity. GSE was found to have a > 30% amino acid homology to *rfbT* of *V.cholerae*, involved in the transport of specific LPS components across the cell membrane. In *V.cholerae* the gene product causes a seroconversion from the Inaba to the Ogawa 'epidemic' strain.  
20 GSF encoded a protein of 30.2kDa which was homologous in the range 25-40% at the amino acid level to several glucosyl transferases such as *rfpA* of *K.pneumoniae*, *rfbB* of *K.pneumoniae*, *lgtD* of *H.influenzae*, *lsi* of *N.gonorrhoeae*. In *E.coli* an equivalent gene *galE* adds  $\beta$ -1-3 N-acetylglucosamine to galactose,  
25 the latter only found in 'O' and 'M' antigens which are also related to pathogenicity. GSH comprising the ORFs GSH<sub>1</sub> and GSH<sub>2</sub> encodes a protein totalling about 60kDa which is a putative transposase with a 40 - 43% homology at the amino acid level to the equivalent gene product of IS21 in *E.coli*. This family of  
30 insertion sequences is broadly distributed amongst gram negative bacteria and is responsible for mobility and transposition of genetic elements. An IS21-like element in *B.fragilis* is split either side of the  $\beta$ -lactamase gene controlling its activation and expression. We programmed an *E.coli* S30 cell-free extract  
35 with plasmid DNA containing the ORF GSH under the control of a *lac* promoter in the presence of a <sup>35</sup>S-methionine, and demonstrated the translation of an abundant 60kDa protein. The proteins homologous to GS encoded in other organisms are in general highly antigenic. Thus the proteins encoded by the ORFs

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in GS may be used in immunoassays of antibody or cell mediated immuno-reactivity for diagnosing infections caused by mycobacteria, particularly *Mptb*, *Mavs* and *Mtb*. Enhancement of host immune recognition of GS encoded proteins by vaccination

5 using naked specific DNA or recombinant GS proteins, may be used in the prevention and treatment of infections caused by *Mptb*, *Mavs* and *Mtb* in humans and animals. Mutation or deletion of all or some of the ORFs A to H in GS may be used to generate attenuated strains of *Mptb*, *Mavs* or *Mtb* with lower pathogenicity

10 for use as living or killed vaccines in humans and animals. Such vaccines are particularly relevant to Johne's disease in animals, to diseases caused by *Mptb* in humans such as Crohn's disease, and to the management of tuberculosis especially where the disease is caused by multiple drug-resistant organisms.

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## SEQUENCE LISTING

Seq. ID No.1

5'- 1 GATCCAACTA AACCCGATGG AACCCCGCGC AAATATTGG ACGTCTCCGC GCTACGCAGT  
 61 TGGGTGGCG CCCGCGAATC GCACTGAAAG AGGGCATCGA TGCAACGGTG TCGTGGTACC  
 121 GCACAAATGC CGATGCCGTG AGGAGGTAAA GCTGCGGGCC GGCCGATGTT ATCCCTCCGG  
 181 CCGGACGGGT AGGGCGACCT GCCATCGAGT GGTACGGCAG TCGCCTGGCC GCGGAGGCGC  
 241 ATGGCCTATG TGAGTATCCC ATAGCCTGGC TTGGCTCGCC CCTACGCATT ATCAGTTGAC  
 301 CGCTTTCGCG CCACGTCGCA GGCTTGCGGC AGCATCCCGT TCAGGTCTCC TCATGGTCCG  
 361 GTGTGGCAGC ACCACGCAAG CTCGAACCGA CTCGTTTCCC AATTTCGCAT GCTAATATCG  
 421 CTCGATGGAT TTTTGGCGA ACGCCGGCTT GATGGCTCGT AACGTTAGCA CCGAGATGCT  
 481 GCGCCACTCC GAACGAAAGC GCCTATTAGT AAACCAAGTC GAAGCATACG GAGTCAACGT  
 541 TGTATTGAT GTCGGTGCTA ACTCCGGCCA GTTCGGTAGC GCTTTGCGTC GTGCAGGATT  
 601 CAAGAGCCGT ATCGTTTCTT TGAACCTCT TTCGGGGCCA TTTGCGCAAC TAACGGGCAA  
 661 GTCGGCATCG GATC -3'

15 Seq. ID No.2

5'- 1 GATCCGATGC CGACTTGCGC GTTAGTTGCG CAAATGGCCC CGAAAGAGGT TCAAAGGAAA  
 61 CGATACGGCT CTTGAATCCT GCAOGACGCA AAGCGCTACC GAACTGGCCG GAGTTAGCAC  
 121 CGACATCAAT AACAAAGTTG ACTCCGTATG CTTGCACTTG GTTTACTAAT AGGCGCTTTC  
 181 GTTCGGAGTG GCGCAGCATC TCGGTGCTAA CGTTACGAGC CATCAAGCCG GCGTTGCGCA  
 241 AAAAATCCAT CGAGCGATAT TAGCATGCGA AATTGGGAAA CGAGTCGGTT CGAGCTTGCG  
 301 TGGTCGTGCC ACACCGGACC ATGAGGAGAC CTGAACGGGA TGCTGCCGCA AGCCTGCGAC  
 361 GTGGCGCGAA AGCGGTCAAC TGATAATGCG TAGGGGCGAG CCAAGCCAGG CTATGGGATA  
 421 CTCACATAGG CCATGCGCCT CGCCGGCCAG GCGACTGCCG TACCACTCGA TGGCAGGTG  
 481 CCCTACCCGT CCGGCCGGAG GGATAACATC GGCCGGCCCG CAGCTTTACC TCCTCACGGC  
 541 ATCGGCATTG GTGCGGTACC ACGACACCGT TGCATCGATG CCCTCTTTCA GTGCGATTG  
 601 CCGGCGCCAA CCCAACTGCG TAGCGCGGAG ACGTCCAATA GTTTGCGCGG GGTTCATCG  
 661 GGTTTAGTTG GATC -3'

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Seq. ID No.3

1 GAATTCTGGG TTGAGACGA CGTCGAACTC CTGGTCGGTC TTGCTTGGAA  
51 TGATCGCTGT GATCTGGTCG GCGGTGCCGA CAGGAACCGT CGACTTGTGG  
101 ACGATCACCT TGTACCGGTC GATGTATGAC CCAATGTGGT CCGCAACCGA  
5 GAAGACGTAC GTCAGGTCCG CCGCCCGCT TTCACCCATG GCGGTGCGGA  
151 CGGCGATGAA AATGACGTCC GCGTCTCGA TTCCGCGTTG CCGGTGCGGTG  
201 GTGAAGTCAA TCAGCCCGTT CTCACGGTTC CTCGCAATCA ACTCCCAACC  
251 CGGGCTCGAA AATCGGGACA CTGCTGCGA GGAGCAAATC GATCTTGGCC  
301 TGATCGATAT CGACACAGAC GACATCGTTG CGCTATCCG CGAGACAAGC  
351 GCCCGTGACG AGGCCTACAT AGCCTGATCC GACCACCGAA ATTTTCAAGA  
10 401 TGACCCCTTC AAGTCCCGA TCGGTGACG ACCATACTGC CGCAACTCTG  
451 TACCTCCGT GGGTAATTG CATGTGCGT TCGTAAGGAG CAGCCAGCGA  
501 GTCGGGACG TTCGGTGAGA GAGTCGAGG ACTACGAGGT TCGCGGTGCG  
551 ATACATCACA GTGTTGCGTC TGTGCGCAAC GATGCAACAA GAACCCACGG  
601 GGCAGCCCTG AACTGCGCGC ATGACCGGTC CTTGTCTGCG CACCTTTGAT  
15 651 CCGCCACCGC TTCCATGCGA ACATGACCGG AATCCATAGC GCGTGGTCAA  
701 GCAGCGGGGA GGTAGACGTC GGTGTCATCT GCTCCAACCG TGTGCGTGAT  
751 AACGATTTG CTGAACGATC TCGAGGGATT GAAAGCACC GTGGAGAGCG  
801 TTCGCGCGCA GCGCTATGGG GGGCGAATCG AGCACATCGT CATCGACCGT  
851 GGATCGGGCG ACGCGTGGT GAGTATCTG TCCGGCGATC CTGGCTTTGC  
20 901 ATATTGGCAA TCTCAGCCCG ACAACGGGAG ATATGACGCG ATGAATCAGG  
951 GCATTGCCCC TTCGTGCGGC GACCTGTTGT GGTTTATGCA CTCACCGGAT  
1001 CGTTTCTCCG ATCCAGATGC AGTCGCTTCC GTGGTGAGAG CGCTCTCGGG  
1051 GCATGGACCA GTACGTGATT TGTGGGTTA CCGGAAAAAC AACCTTGTGG  
1101 GACTCGACGG CAAACCACTT TTCCCTCGGC CGTACGGCTA TATGCCGTTT  
25 1151 AAGATGCGGA AATTTCTGCT CCGCGCGACG GTTGCGCATC AGGCGACATT  
1201 CTTGCGCGCG TCGCTGGTAG CCAAGTTGGG CGGTTACGAT CTTGATTTTG  
1251 GACTCGAGGC GGACCACTG TTCATCTACC GTGCCGCACT AATACGGCCT  
1301 CCCGTCAAGA TCGACCGCGT GGTTTGCGAC TTCGATGTCA CCGGACCTGG  
1351 TTCAACCCAG CCCATCCGTG AGCACTATCG GACCTGCGG CGGCTCTGGG  
30 1401 ACCTGCATGG CGACTACCG CTGGGTGGG GCAGAGTGTC GTGGGCTTAC  
1451 TTGCGTGTGA AGGAGTACTT GATTCGGGCC GACCTGGCCG CATTCAACGC  
1501 GGTAAAGTTC TTGCGAGCGA AGTTCGCCAG AGCTTCGCGG AAGCAAAATT  
1551 CATAGAAACC AACTTCTACT GCCTGACCTG AGCAGCGCCG AGGCGGCGAG  
1601 CCGGATCAGT GCGACCTGAA CCGCCAGGTG GAAAGCGCCA CCGATCCCGG  
35 1651 CACCGAGTGC CTGACGCTTC GGATCCCTTG CACCACAACG AGAGTGAGAG  
1701 CGCCATGATG AGGAAATATC GGCTGGGCGG AGTCAACGCC GGAGTGACAA  
1751 AAGTGAGAAC CCGGTGAAGC GAGCGCTTAT AACAGGGATC ACGGGGCAGG  
1801 ATGGTTCCTA CCTCGCCGAG CTACTACTGA GCAAGGGATA CGAGGTTTAC  
1851 GGGCTCGTTC GTCGAGCTTC GACGTTTAAAC ACGTCGCGGA TCGATCACCT  
40 1901 CTACGTTGAC CCACACCAAC CCGGCGCGCG CTGTTCTTG CACTATGCAG  
1951 ACCTCACTGA CGGCACCCGG TTGGTGACCC TGCTCAGCAG TATCGACCCG  
2001 GATGAGGTCT ACAACCTCGC AGCGCAGTCC CATGTGCGCG TCAGCTTTGA  
2051 CGAGCCAGTG CATACCGGAG ACACCAACCG CATGGGATCG ATCGACTTC  
2101 TGGGAAGCAGT CCGCCTTTCT CCGGTGGACT GCCGGTTCTA TCAGGCTTCC  
45 2151 TCGTCGGAGA TGTTCGGCGC ATCTCGCCA CCGCAGAACG AATCGAAGCC  
2201 GTTCTATCCC CGTTCGCCAT ACGGCGCGGC CAAGGTCTTC TCGTACTGGA  
2251 CGACTCGCAA CTATCGAGAG GCGTACGGAT TATTGCGAGT GAATGGCATC  
2301 TTGTTCAACC ATGAGTCCCC CCGGCGCGGC GAGACTTTTC TGACCCGAAA  
2351 GATCAGCGGT GCCGTGGCGC GCATCCGAGC TGGCGTCCAA TCGGAGGTCT  
50 2401 ATATGGGCAA CCTCGATGCG ATCCGCGACT GGGGCTACGC GCCCGAATAT  
2451 GTCGAGGGGA TGTGGAGGAT GTTGCAAGCG CCTGAACCTG ATGACTACGT  
2501

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5 2551 CCTGGCGACA GGGCGTGGTT ACACCGTACG TGAGTTCGCT CAAGCTGCTT  
2601 TTGACCATGT CGGGCTCGAC TGGCAAAAGC GCGTCAAGTT TGACGACCGC  
2651 TATTTGCGTC CCACCGAGGT CGATTGCTA GTAGGAGATG CCGACAAGGC  
2701 GGCCCACTCA CTCGGCTGGA AAGCTTCGGT TCATACTGGT GAAGTCGCGC  
2751 GCATCATGGT GGACGCGGAC ATCGCGCGT TGGAGTGOA TGGCACACCA  
10 2801 TGGATCGACA CGCCGATGTT GCCTGGTTGG GGCAGAGTAA GTTGACGACT  
2851 ACACCTGGGC CTCTGGACCG CGCAACGCCC GTGTATATCG CCGGTCATCG  
2901 GGGGCTGGTC GGCTCAGCGC TCGTACGTAG ATTTGAGGCC GAGGGGTTCA  
2951 CCAATCTCAT TGTGCGATCA CGCGATGAGA TTGATCTGAC GGACCGAGCC  
10 3001 GCAACGTTTG ATTTTGTGTC TGAGACAAGA CCACAGGTGA TCATCGATGC  
3051 GGCCGACCGG GTGGGCGCA TCATGGCGAA TAACACCTAT CCGCGGACT  
3101 TCTTGTCCGA AAACCTCCGA ATCCAGACCA ATTTGCTCGA CGCAGCTGTC  
3151 GCCGTGCGTG TGCCGCGGCT CCTTTTCCTC GGTTCTCAT GCATCTACCC  
15 3201 GAAGTAGCT CCGCAACCTA TCCACGAGAG TGCTTTATTG ACTGGCCCTT  
3251 TGGAGCCAC CAACGACCGG TATGCGATCG CCAAGATCGC CGGTATCCTG  
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3351 GCCGACTAAC CTCTACGGAC CCGGCGACAA CTCTCCCCG TCCGGGTGCG  
3401 ATCTCTTGCC GGCCTCATC CGTCGATATG AGGAAGCCAA AGCTGGTGGT  
3451 GCAGAAGAGG TGACGAATTG GGGGACCGGT ACTCCGCGG CCGAACTTCT  
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3601 GAGATCGCAG ACATGGTCGC TACAGCGGTG GGCTACATCG GCGAAACACG  
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25 3701 CCGCGCTACG CGAGTTGGGT TGGGCGCCG GAATCGCACT GAAAGACGGC  
3751 ATCGATGCAA CGGTGTCGTG GTACCGCACA AATGCGATG CCGTGAGGAG  
3801 GTAAAGCTGC GGGTCGGCG ATGTTATCCC TCCGGCCGGA CCGGTGGGGC  
3851 GACCTGCCGT CGAGTGGTAC GGCAGTCGCC TGGCCGGCGA GCGCGGTGGC  
3901 CTATGGAGT ATCCAATAGC CTGGCTTGGC TCGCCCTAC GCATTATCAG  
3951 TTGACCGCTT TCGCGCCAGC TCGCAGGCTT GCGGCAGCAT CCGTTCAGG  
30 4001 TCTCCTCATG GTCCGGTGTG GCACGACCAC GCAAGCTCGA ACCGACTCGT  
4051 TTCCCAATTT CGCATGCTAA TATCGCTCGA TGGATTTTTT GCGCAACGCC  
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4151 AAAGCGCCTA TTAGTAAACC AATTCAAAGC ATACGGAGTC AACGTTGTTA  
4201 TTGATGTCGG TGCTAACTCC GGCCAGTTCC GTAGCGCTTT GCGTCGTGCA  
35 4251 GGATTCAAGA GCCGTATCGT TTCCTTTGAA CCTCTTTCGG GGCCATTGTC  
4301 GCAACTAAC CGCAAGTCGG CATCGGATCC ACTATGGGAG TGTCAACAGT  
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40 4501 TTGATTCGGT TGCATCAGAA TTTCTGAACC CTACGATGT TACTTTCTG  
4551 AAGATCGACG TACAGGGTTT CGAGAAGCAG GTTATCACGG GCAGTAAGTC  
4601 AACGCTTAAC GAAAGCTGCG TCGGCATGCA ACTCGAACTT TCTTTTATTC  
4651 CGTTGTACGA AGGTGACATG CTGATTATG AAGCGCTTGA ACTTGTCTAT  
4701 TCCCTAGGTT TCAGACTGAC GGGTTTGTG CCGGCTTTA CCGATCCGCG  
45 4751 CAATGGTCGA ATGCTTCAAG CTGACGGCAT TTTCTTCCGT GGGGACGATT  
4801 GACATAAATG CTCCTGCGC ACCCTGCGG TATCCAAACG GCGATCTGG  
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4951 CTGCGCCAGT GTTCTCGATA ATTATCCCTA CCTTCAATGC AGCGGTGAAG  
50 5001 CTGCAAGCCT GCCTCGGAAG CATCGTCGGG CAGACCTACC GGGAAAGTGA  
5051 AGTGGTCCTT GTCGACGGCG GTTCGACCGA TCGGACCCCTC GACATCGCGA  
5101 ACAGTTTCCG CCCGGAATC GGCTCGCGAC TGGTCGTTCA CAGCGGGCCC  
5151 GATGATGGCC CCTAGGACGC CATGAACCGC GCGCTCGGCG TGGCCACAGG

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5201 CGAATGGGTA CTTTTTTTAG GCGCOGACGA CACCCCTCTAC GAACCAACCA  
 5251 CGTTGGCCCA GGTAGCCGCT TTTCTCGGCG ACCATGCGGC AAGCCATCTT  
 5301 GTCTATGGCG ATGTTGTGAT GCGTTCGACG AAAAGCCGGC ATGCCGGACC  
 5351 TTTCGACCTC GACCGCTCC TATTTGAGAC GAATTTGTGC CACCAATCGA  
 5401 TCTTTTACCG CCGTGAGCTT TTCGACGGCA TCGGCCCTTA CAACCTGCGC  
 5451 TACCGAGTCT GGGCGGACTG GGACTTCAAT ATTCTGCTGCT TCTCCAACCC  
 5501 GCGGCTGATT ACCCGCTACA TGGACGTGCT GATTTCCGAA TACAACGACA  
 5551 TGACCGGCTT CAGCATGAGG CAGGGGACTG ATAAAGAGTT CAGAAAACGG  
 5601 CTGCCAATGT ACTTCTGGGT TGCAGGGTGG GAGACTTGCA GGCGCATGCT  
 5651 GCGGTTTTTG AAAGACAAGG AGAATCGCCG TCTGGCCTTG CGTACGCGGT  
 5701 TGATAAGGGT TAAGGCCGTC TCCAAAGAAC GAAGCGCAGA ACCGTAGTCG  
 5751 CGGATCCACA TTGGACTTCT TTAACGCGTT TCGCTCCTGA TCCACCTTTC  
 5801 AAGCCCGTTC CGCGTAACGC GCGCGCAGA GAGTGGTCGC ATATCGCATC  
 5851 ACTGTTCTCG TGCCAGTGCT TGGAAAGCGT CGAGCACTCT GGTTCGGGTT  
 5901 CTTGACGTTT GCGCCCGCTC CTAGAGGTAG CGTGTCAGT GACTGAAGCC  
 5951 AATGAGTGCA ACTCGCGTC GCGAAAGGTT TCAGTCCGGG TTGAGCAAGA  
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 6051 AAAGCGGATG CAAAGGGATT CGAAGCTTGA GCAACATGCG AAGGGGAGAA  
 6101 CGGCCTATGA GGCTGGGACA GGTTTTCGAT CCGCGCGCGA ATGCACTGTC  
 6151 AATGGCCAAG TAGAAGTCCC CGCTGGTGGC CAGCAGAAGT CCCCACCTCG  
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 6451 GCGGGGCGAA GCCGATCTCG TCCAAGATGA CCAGATCCGC GCGGAGCAGG  
 6501 GTGTGATGA TCTTCCGAC GGTGTTGTCG GCCAGGCCGC GGTAGAGGAC  
 6551 CTCGATCAGG TCGGCGGCGG TGAAGTAGCG GACTTTGAAT CCGGCGTGGA  
 6601 CCGCAGCGTG CCGCAGCCG ATGAGCAGGT GACTTTTGCC CGTACCAGGT  
 6651 GGGCCAATGA CCGCCAGGTT CTGTTGTGCC CGAATCCATT CCAGGCTCGA  
 6701 CAGGTAGTCG AACGTGGCTG CGGTGATCGA CGATCCGGTG ACGTCGAACC  
 6751 CGTCGAGGGT CTTGGTGACC GGGAAAGGCTG CCGCCTTGAG ACGGTTGGCG  
 6801 GTGTTGGAGG CATCGCGGGC AGCGATCTCG GCCTCAACCA ACGTCCGCG  
 6851 GATCTCCTCC GGTGTCCAGC GTTGCCTCTT GGCGACTTGC AACACCTCGG  
 6901 CCGCGTTGCG GCGCACCGTG GCCAGCTTCA ACCGCGCGAG CGCGCGTCA  
 6951 AGGTCAGCAG CCAGCGGTGC CGCCGAGGAC GGTGCCACCG GCTTGGCAGC  
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 7051 CGAGCGGTC TCGACGGTGG GCAGATCGAG CACGAGTGCG TCGCCGCGG  
 7101 GCGGGGTTG TGGGTGCGG GCGCCGCGG CCAGGATCGA GCGCACGTGG  
 7151 GCAGCGCGGA ACCGGCGAAA CGCAACCGCC CCGCGCAGCG CGTCAATCAA  
 7201 AGCCTGTTCC CCGTGGGCGG CGCCAAGGCC GAGCAGAAATG TCGAGTTCCG  
 7251 ATTTCACTCG GGTGTTGCCG ATCGCAGCAG CACCGACGAG GAACTGCTGC  
 7301 GCTTCGGTTC CCAATGCGCA GAATCGTTTC TCTGCTTGGG TTTTCGGGCG  
 7351 AGGACCACGC GAGGGTGCGG GTCTGGGTCC GTCGTAGTGT TCATCGAGGA  
 7401 TGGACACCTC ACCTGGGCTG ACGAGCTCGT GCTCGGCCAC GATCACACCG  
 7451 GTCGCAGGTT CCAACAGGAT CAGGGCGCCA TGATCGACCA CCACCGCCAC  
 7501 GGTGGCACCG ACGAGCCGCT GAGGCACCGA GTAACGAGCT GAGCCGTAAC  
 7551 GGATGCACGA GAGGCCGTGG ACCTTACGCG GCACCGACCC CGAGCCGATC  
 7601 GTCGGCCGCA GCGAGGGCAG CTCCTCAAG ACGGTGCGCT CGTCAACCAA  
 7651 GCGATCGTTG GGCACGGGCG AGATCTCCGA GTGGACCGTG GCATTGACCT  
 7701 CCGCGCACCA TAGTTGCGCC TGGGCGTTGA GGGCAGTAG GTCGACCTGC  
 7751 TCACCGGCTA ACGCAGCTTC GGTGAGCAGC GGCACCGCAA GGTCGTCTGT  
 7801 AGCGTAGCCA CAGAGGTTCT CCAGGATGCC CTTCGATTGC GGATCCGCAC

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7851 CGTGGCAGAA GTCCGGAACG AAGCCATAGT GGGACGCGAA TCGCACATAA  
 7901 TCCGGTGTG GAACAACAAC ATTGGCGACG ACACCACCTT TGAGGCAGCC  
 7951 CATCCGGTCG GCCAGGATCT TGGCCGGAAC CCCACCGATC GCCTC

Seq. ID No. 4

5 1 TTCTACTGCC TGACCTGAGC AGCGCCGAGG CGCGCAGCGC GATCACTGCG ACCTGAATGG  
 61 CCAGGTGGAA AGCGCCACCG ATCCCGGCAC CGAGTGCCTG ACGATTCGGA TCCCTTGACAC  
 121 CACAACGAGA GTGAGACCGC CATGATGACG AAATATCGGC TGGGCGGAGT CAACGCGGGA  
 181 GTGACAAAAG TGAGAACCAG GTGAAGCGAG CGCTTATAAC AGGGATCACG GGGCAGGATG  
 241 GTTCCTACCT CGCCGAGCTA CTACTGAGCA AGGGATACGA GGTTCACGGG CTGGTTGGTC  
 10 301 GAGCTTCGAC GTTTAACACG TCGCGGATCG ATCACCTCTA CGTTGACCCA CACCAACCGG  
 361 GCGCGCGCTT GTTCTTGAC TATGCAGACC TCACTGACGG CACCGGGTTG GTGACCTGCG  
 421 TCAGCAGTAT CGACCCGGAT GAGGTCTACA ACCTCGCAGC GCAGTCCCAT GTGCGCGTCA  
 481 GCTTTGACGA GCCAGTGCAAT ACCGAGAGCA CCACCGGCAT GGGATCGATC CGACTTCTGG  
 541 AAGCAGTCCG CCTTTCTCGG GTGGACTGCC GGTTCCTATCA GGCTTCCTCG TCGGAGATGT  
 15 601 TCGGCGCATC TCCGCCACCG CAGAACGAAT CGACGCGCTT CTATCCCGT TCGCCATACG  
 661 GCGCGGCCAA GGTCTTCTCG TACTGGACGA CTCGAACTA TCGAGAGGCG TACGGATTAT  
 721 TCGCAGTGAA TGGCATCTTG TTCAACCATG AGTCCCCCGG GCGCGCGCAG ACTTTCTGTA  
 781 CCCGAAAGAT CACGCGTGCC GTGGCGCGCA TCCGAGCTGG CGTCCAATCG GAGGTCTATA  
 841 TGGGCAACCT CGATGCGATC CGGACTGGG GCTACGCGCC CGAATATGTC GAGGGGATGT  
 20 901 GGAGGATGTT GCAAGCGCCT GAACCTGATG ACTACGTCTT GCGCAGAGGG CGTGGTTACA  
 961 CCGTACGTGA GTTCGCTCAA GCTGCTTTTG ACCACGTGGG GCTCGACTGG CAAAAGCACG  
 1021 TCAAGTTTGA CGACCGCTAT TTGCGCCCCA CCGAGGTGGA TTGCTAGTA GGAGATGCCG  
 1081 ACAGGGCGGC CCAGTCACTC GGCTGGAAAG CTTGCGTTCA TACTGGTGAA CTCGCGCGCA  
 1141 TCATGGTGGA CGCGGACATC GCGCGTGGG AGTGCGATGG CACACCATGG ATCGACACGC  
 25 1201 CGATGTTGCC TGGTTGGGGG GGAGTAAGTT GACGACTACA CCTGGGCTCT TGGACCGCGC  
 1261 AACGCCCGTG TATATCGCGG GTCATCGGGG GCTGGTGGG TCAGCGCTCG TACGTAGATT  
 1321 TGAGGCCGAG GGGTTCACCA ATCTCATTGT GCGATCACGC GATGAGATTG ATCTGACGGA  
 1381 CCGAGCCGCA ACGTTTGATT TTGTGTCTGA GACAAGACCA CAGGTGATCA TCGATGCGGC  
 1441 CGCACGGGTC GCGCGCATCA TGGCGAATAA CACCTATCCC GCGGACTTCT TGTCCGAAAA  
 30 1501 CCTCCGAATC CAGACCAATT TGCTCGACGC AGCTGTGCGC GTGCGTGTGC CGCGGCTCCT  
 1561 TTTCTCTGGT TCGTCATGCA TCTACCCGAA GTACGCTCCG CAACCTATCC ACGAGAGTGC  
 1621 TTTATTGACT GGCCCTTTGG AGCCACCAAA CGACGCGTAT GCGATCGCCA AGATCGCCCG  
 1681 TATCTGCAA GTTCAGGCGG TTAGGCGCCA ATATGGGCTG GCGTGGATCT CTGCGATGCC  
 1741 GACTAACCTC TACGGACCGG GCGACAACTT CTCCCGTCC GGGTGGCATC TCTTGCCGCG  
 35 1801 GCTCATCCGT CGATATGAGG AAGCCAAAGC TGGTGGTGCA GAAGAGGTGA CGAATTGGGG  
 1861 GACCGGTACT CCGCGGCGCG AACTTCTGCA TGTGACGAT CTGGCGAGCG CATGCCCTGT  
 1921 CCTTTTGAA CATTTCGATG GTCGAAACCA CGTCAACGTG GGCACCGGGG TCGATCACAG  
 1981 CATTAGCGAG ATCGCAGACA TGGTCGCTAC GCGGTTGGG TACATCGGCG AAACACGTTG  
 2041 GGATCCAACT AAACCCGATG GAACCCCGCG CAAACTATTG GACGTCTCCG CGCTAEGCGA  
 40 2101 GTTGGGTTGG CGCCCGCGAA TCGCACTGAA AGACGGCATC GATGCAACGG TGTGTTGGTA  
 2161 CCGCACAAAT GCCGATGCCG TGAGGAGGTA AAGCTGCGGG CCGGCGGATG TTATCCCTCC  
 2221 GGCGGACGCG GTAGGGCGAC CTGCCATCGA GTGGTACGGC AGTCCGCTGG CCGGCGAGGC  
 2281 GCATGGCCTA TGGGAGTATC CCAATAGCTG GCTTGGCTCG CCCCTACGCA TTATCAGTTG  
 2341 ACCGCTTTG CGCCAGCTCG CAGGCTCGCG GCAGCATCCC GTTCAGGTCT CCTCATGGTC  
 45 2401 CGGTGTGGCA CGACCACGCA AGCTCGAACC GACTCGTTTC CCAATTTGGC ATGCTAATAT  
 2461 CGCTCGATGG ATTTTGTGCG CAACGCGGGC TTGATGGCTC GTAAAGTTAG CACCGAGATG  
 2521 CTGCGCCACT TCGAACGAAA GCGCCTATTA GTAAACCAAT TCAAAGCATA CGGAGTCAAC  
 2581 GTTGTATTG ATGTCGGTGC TAACTCGGGC CAGTTGCGTA GCGCTTTGG TCGTGCAGGA  
 2641 TTCAAGAGCC GTATCGTTTC CTTTGAACCT CTTTGGGGC CATTTGCGCA ACTAACGCGC  
 50 2701 GAGTCGGCAT CGGATCCACT ATGGGAGTGT CACCAAGTATG CCTAGGGGGA CGCGGATGAG

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2761 ACGATTACCA TCAATGTGGC AGGCAATGCG GGGGCAAGTA GTTCGGTGCT GCCGATGCTT  
 2821 AAAAGTCATC AAGATGCCTT TCCTCCGCG AATTATATTG GCACCGAAGA CGTTGCAATA  
 2881 CACCGCCTTG ATTCGGTTGC ATCAGAATTT CTGAACCCTA CCGATGTTAC TTTCTGGAAG  
 2941 ATCGACGTAC AGGGTTTCGA GAAGCAGGTT ATCGCGGGCA GTAAGTCAAC GCTTAACGAA  
 5 3001 AGCTGCGTCG GCATGCAACT CGAACTTTCT TTTATTCCGT TGTACGAAGG TGACATGCTG  
 3061 ATTCATGAAG CGCTTGAAC TGTCTATTCC CTAGGTTTCA GACTGACGGG TTTGTTGCCC  
 3121 GGATTTACGG ATCCGCGCAA TGGTCGAATG CTTCAAGCTG ACGGCATTTT CTTCCGTGGG  
 3181 GACGATTGAC ATAAATGCTT GCGTCGGCAC CCTGCCGTA TCCAAACGGG CGATCTGGTG  
 3241 AGCCGGCCTC CCGGGCACCT AATCGACTAT CTAAATTGAG GCGGCCGCGA CGTGCGGCAC  
 10 3301 GAACAGGTGG CCGGCTGCTA GCGTTACACA CGTCATGACT GCGCCAGTGT TCTCGATAAT  
 3361 TATCCCTACC TTCAATGCAG CCGTGACGCT GCAAGCCTGC CTCGGAAGCA TCGTCGGGCA  
 3421 GACCTACCGG GAAAGTGAAG TGGTCCTTGT CGACGGCGGT TCGACCGATC GGACCCTCGA  
 3481 CATCGGAAC AGTTTCCGCC CGAACTCGG CTCGCGACTG GTGTTTACA GCGGGCCCGA  
 3541 TGATGGCCCC TACGACGCCA TGAACCGCGG CGTCGGCGTA GCCACAGGCG AATGGGTACT  
 15 3601 TTTTTTAGGC GCCGACGACA CCTCTACGA ACCAACCACG TTGGCCAGG TAGCCGCTTT  
 3661 TCTCGGCGAC CATGCGGCAA GCCATCTTGT CTATGGCGAT GTTGTGATGC GTTCGACGAA  
 3721 AAGCCGGCAT GCCGGACCTT TCGACCTCGA CCGCCTCCTA TTTGAGACGA ATTTGTGCCA  
 3781 CCAATCGATC TTTTACGCC GTGAGCTTTT CGACGGCATC GGCCCTTACA ACCTGCGCTA  
 3841 CCGAGTCTGG GCGGACTGGG ACTTCAATAT TCGCTGCTTC TCCAACCCGG CGCTGATTAC  
 20 3901 CCGCTACATG GACGTCGTGA TTTCTGAATA CAACGACATG ACCGGCTTCA GCATGAGGCA  
 3961 GGGGACTGAT AAAGAGTTCA GAAACCGCT GCCAATGTAC TTCTGGGTTG CAGGGTGGGA  
 4021 GACTTGCAAG CGCATGCTGG CGTTTTTGAA AGACAAGGAG AATCGCCGTC TGGCCTTGCG  
 4081 TACGCGGTTG ATAAGGGTTA AGGCCGTCTC CAAAGAACGA AGCGCAGAAC CGTAGTCCGG  
 4141 GATCCACATT GGACTTCTTT AACCGGTTTG CGTCTGATC CACCTTTCAA CCCCGTTCCG  
 25 4201 CGTGACGCGG CGCGCAGAGA GTGGTCGCAT ATCGCGTCAC TGTCTCTGTG CCAGTGCTTG  
 4261 GAAAGCGTCG AGCACTCTGG TTCGCGTCTT TGACGTTTCG GCCCGCCCTT AGAGGTAGCG  
 4321 TGTCACTGTA CTGAAGCCAA TGAGTGAAC TCGGCGTCGC GAAAGGTTTC AGTCGCGGTT  
 4381 GAGCAAGACA CCGCAAGACT ACTGGAGTGC GTGCACAAGC GCCTCCAGCT CACGG

## Seq. ID No.5

30 1 atgatcgctg tgatctggtc ggcggtgccg acaggaaccg tcgacttgct gacgatcacc  
 61 ttgtaccggt cgatgtatga cccaatgtcg tccgcaaccg agaagacgta cgtcagggtcc  
 121 gccgccccgc tttcaccat gggcgctcgg acggcgatga aaatgacgct cgcgtgctcg  
 181 attcgcggtt gccggtcggg ggtgaagtca atcagcccg tctcacggtt cctcgcaatc  
 241 aactcccaac ccgggctcga aaatcgggac actgcctgcg aggagcaaat cgatcttggc  
 35 301 ctgatcgata tcgacacaga cgacatcgtt gccgctatcc gcgagacagg cgcccgtagc  
 361 gaggcctaca tagcctga

## Seq. ID No.6

40 1 M I A V I N S A V P T G T V D L S T I T L Y R S M Y D P M S  
 31 S A T E K T Y V R S A A P L S P M G V G T A M K M T S A C S  
 61 I P R C R S V V K S I S P F S R P L A I N S Q P G L E N R D  
 91 T A C E E Q I D L G L I D I D T D D I V A A I R E T G A R D  
 121 E A Y I A

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## Seq. ID No.7

1 gtgtcatctg ctccaaccgt gtcggtgata acgatttcgc tgaacgatct cgagggattg  
61 aaaagcaccg tggagagcgt tcgcgcgcag cgctatgggg ggcgaatcga gcacatcgte  
121 atcgacgggtg gatcgggcga cgccgtcgtg gagtatctgt ccggcgatcc tggctttgca  
5 181 tattggcaat ctccagcccg caacgggaga tatgacgcga tgaatcaggg cattgcccat  
241 tcgtcggggc accctgttggt gtttatgcac tccacggatc gtttctccga tccagatgca  
301 gtcgcttccg tggtaggggc gctctcgggg catggaccag tacgtgattt gtgggggttac  
361 gggaaaaaca accctgtcgg actcgacggc aaaccacttt tccctcggcc gtacggctat  
421 atgccgttta agatgcggaa atttctgctc ggcgcgacgg ttgcgcacga ggcgacattc  
10 481 ttcggcgcggt cgctggtagc caagttgggc gggtacgac ttgattttgg actcgaggcg  
541 gaccagctgt tcctctaccg tgcgcacta atacggcctc ccgtcacgat cgaccgcgtg  
601 gtttgcgact tcgatgtcac gggacctgggt tcaaccacgc ccatccgtga gcactatcgg  
661 accctgcggc ggctctggga cctgcatggc gactaccgcg tgggtggggc cagagtgtcg  
721 tgggcttact tgcgtgtgaa ggagtacttg attcggggcg accctggccg attcaacgcg  
15 781 gtaagttct tgcgagcgaa gttcgccaga gtttcgcgga agcaaaattc atag

## Seq. ID No.8

1 V S S A P T V S V I T I S L N D L E G L K S T V E S V R A Q  
31 R Y G G R I E H I V I D G G S G D A V V E Y L S G D P G F A  
61 Y W Q S O P D N G R Y D A M N Q G I A H S S G D L L W F M H  
20 91 S T D R F S D P D A V A S V V E A L S G H G P V R D L W G Y  
121 G K N N L V G L D G K P L F P R P Y G Y M P F K M R K P L L  
151 G A T V A H Q A T F F G A S L V A K L G G Y D L D F G L E A  
181 D Q L F I Y R A A L I R P P V T I D R V V C D F D V T G P G  
211 S T Q P I R E H Y R T L R R L W D L H G D Y P L G G R R V S  
25 241 W A Y L R V K E Y L I R A D L A A F N A V K F L R A K P A R  
271 A S R K Q N S

## Seq. ID No.9

1 gtgaagcgag cgcttataac agggatcacg gggcaggatg gttcctacct cgccgagcta  
61 ctactgagca agggatacga ggttcacggg ctgcgttcgtc gagcttcgac gtttaacacg  
121 tcgcggatcg atcacctcta cgttgaccca caccaaccgg gcgcgcgctt gttcttgca  
30 181 tatgcagacc tcaactgacg caccgggttg gtgacctgc tcagcagtat cgaccggat  
241 gaggtctaca acctcgcagc gcagtcctcat gtgcgcgtca gctttgacga gccagtgc  
301 accggagaca ccaccggcat gggatcgatc cgacttctgg aagcagtcg ctttctcgg  
361 gtggactgcc ggttctatca ggcttctcgc tcggagatgt tcggcgcatc tccgccaccg  
35 421 cagaacgaat cgacgccgtt ctatccccgt tcgccatagc gcgcggccaa ggtcttctc  
481 tactggacga ctgcgaacta tcgagaggcg tacggattat tcgcagtgaa tggcatcttg  
541 ttcaaccatg agtcccccg gcgcggcgag actttcgtga cccgaaagat cagcgtgcc  
601 gtggcgcgca tccgagctgg cgtccaatcg gaggtctata tgggcaacct cgatgcgac  
661 cgcgactggg gctacgcgcc cgaatatgtc gaggggatgt ggaggatgt gcaagcgct  
40 721 gaacctgatg actacgtcct ggcgacggg cgtgggtaca ccgtacgtga gttcgtcaa  
781 gctgcttttg accatgtcgg gctcgactgg caaaagcgcg tcaagttga cgaccgctat  
841 ttgcgtccca ccgaggtcga ttcgctagta ggagatgccg acaaggcgcc ccagtcactc  
901 ggctggaaag cttcggttca tactgggtga ctgcgcgcga tcatggtgga cgcggacatc  
961 gccgcgttgg agtgcgatgg cacaccatgg atcgacacgc cgatgttgcc tgggtggggc  
45 1021 agagtaagtt ga

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## Seq. ID No.10

1 VKRALITGITGQDGSYLAELLLSKGYEVHG  
 31 LVRRASTFNTSRIDHLYVDPHQPGARLFLH  
 61 YADLTDGTRLVTLSSIDPDEVYNLAAQSH  
 91 VRVSFDEP VHTGDTTGMGSI RLL EAVRLSR  
 121 VDCRFYQASSSEMFGASPPPQNESTPFYPR  
 151 SPYGAAKVFSYWTTRNYREAYGLFAVNGIL  
 181 FNHESPRRGETFVTRKITRAVARIRAGVQS  
 211 EYMG NLD AIRDWGYAPBYVEGMWRMLQAP  
 241 EPDDYVLA TGRGYTVREFAAAFDHSVGLDW  
 271 QKR VKFDDRYLRPT EVDSL VGDAADKAAQSL  
 301 GWKASVHTGELARIMVDADIAALECDGTPW  
 331 IDTPNLPGWGRVS

## Seq. ID No.11

1 gtgaagcgag cgcttataac agggatcacg gggcaggatg gttcctacct cgccgagcta  
 61 ctactgagca agggatacga gggttcacggg ctggttcgctc gagcttcgac gtttaacacg  
 121 tcgcggatcg atcacctcta cggttgaccca caccaaccgg gcgcgcgctt gttcttgac  
 181 tatgcagacc tcactgacgg caccgggttg gtgacctgc tcagcagtat cgaccgggat  
 241 gaggtctaca acctcgacgc gcagtcctcat gtgcgcgctca gctttgacga gccagtgcac  
 301 accggagaca ccaccggcat gggatcgatc cgacttctgg aagcagtcgg ctttctcgg  
 361 gtggactgcc gggtctatca gggttctctg tcggagatgt tcggcgcatc tccgccaccg  
 421 cagaacgaat cgacgcccgt ctatccccgt tcgccatacg gcgcggccaa ggtcttctcg  
 481 tactggacga ctgcgaacta tcgagagggc tacggattat tcgcagtgaa tggcatcttg  
 541 ttcaaccatg agtccccccg gcgcggcgag actttctgta cccgaaagat cagcggtgcc  
 601 gtggcgcgca tccgagctgg cgtccaatcg gaggtctata tgggcaacct cgatgcgac  
 661 cgcgactggg gctacgcgcc cgaatatgtc gaggggatgt ggaggatgtt gcaagcgctt  
 721 gaacctgatg actacgtcct ggcgacaggg cgtggttaca ccgtacgtga gttcgctcaa  
 781 gctgcttttg accacgtcgg gctcgactgg caaaagcacg tcaagtttga cgaccgctat  
 841 ttgcgccccca ccgaggtcga ttcgctagta ggagatgccg acagggcggc ccagtcactc  
 901 ggctggaaag ctcggttca tactggtgaa ctgcgcgca tcatggttga cgcggacatc  
 961 gccgcgtcgg agtgcgatgg cacaccatgg atcgacacgc cgatgttgcc tgggtggggc  
 1021 ggagtaagtt ga

## Seq. ID No.12

1 VKRALITGITGQDGSYLAELLLSKGYEVHG  
 31 LVRRASTFNTSRIDHLYVDPHQPGARLFLH  
 61 YADLTDGTRLVTLSSIDPDEVYNLAAQSH  
 91 VRVSFDEP VHTGDTTGMGSI RLL EAVRLSR  
 121 VDCRFYQASSSEMFGASPPPQNESTPFYPR  
 151 SPYGAAKVFSYWTTRNYREAYGLFAVNGIL  
 181 FNHESPRRGETFVTRKITRAVARIRAGVQS  
 211 EYMG NLD AIRDWGYAPEYVEGMWRMLQAP  
 241 EPDDYVLA TGRGYTVREFAAAFDHSVGLDW  
 271 QKHVKFDDRYLRPT EVDSL VGDAADRAAQSL  
 301 GWKASVHTGELARIMVDADIAASECDGTPW  
 331 IDTPMLPGWGGVS

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## Seq. ID No.13

1 gtgcatggc acaccatgga tcgacacgcc gatgttgccct ggttggggca gagtaagttg  
61 acgactacac ctgggcctct ggaccgcgca acgcccgtgt atatcgccg tcacggggg  
121 ctggtcggct cagcgctcgt acgtagattt gaggccgagg ggttcaccaa tctcattgtg  
181 cgatcacgcy atgagattga tctgacggac cgagccgcaa cgtttgattt tgtgtctgag  
241 acaagaccac aggtgatcat cgatgcggcc gcacgggctg gcggcatcat ggcgaataac  
301 acctatcccg cggacttctt gtccgaaaac ctccgaatcc agaccaattt gctcgacgca  
361 gctgtcgccg tgcgtgtgcc gcggctcctt ttcttcggtt cgtcatgcat ctaccggaag  
421 tacgctccgc aacctatcca cgagagtgtt ttattgactg gccctttgga gccaccaaac  
481 gacgcgtatg cgatcgccaa gatcgccggt atcctgcaag ttcaggcggg taggcgcaa  
541 tatgggctgg cgtggatctc tgcgatgccg actaacctct acggaccggg cgacaacttc  
601 tccccgtccg ggtcgcatct cttgccggcg ctcatccgtc gatatgagga agcgaagct  
661 ggtggtgcag aagagggtac gaattggggg accggtactc cgcggcgaga acctctgcat  
721 gtcgacgacg tggcgagcgc atgctgttcc cttttggaac atttcgatgg tccgaaccac  
781 gtcaacgtgg gcaccggcgt cgatcacagc attagcgaga tcgcagacat ggtcgctaca  
841 gcgggtgggt acatcggcga aacacgttgg gatccaacta aaccgagtg aaccgcgc  
901 aaactattgg acgtctccgc gctacgcgag ttgggttggc gcccggaat cgcactgaaa  
961 gacggcatcg atgcaacggt gtcgtgttac cgcacaaatg ccgatgccgt gaggaggtaa

## Seq. ID No.14

1 V R W H T M D R H A D V A W L G Q S K L T T T P G P L D R A  
31 T P V Y I A G H R G L V G S A L V R R F E A E G F T N L I V  
61 R S R D E I D L T D R A A T F D F V S E T R P Q V I I D A A  
91 A R V G G I M A N N T Y P A D F L S E N L R I Q T N L L D A  
121 A V A V R V P R L L F L G S S C I Y P K Y A P Q P I H E S A  
151 L L T G P L E P T N D A Y A I A K I A G I L Q V Q A V R R Q  
181 Y G L A W I S A M P T N L Y G P G D N F S P S G S H L L P A  
211 L I R R Y B E A K A G G A B E V T N W G T G T P R R E L L H  
241 V D D L A S A C L F L L E H F D G P N H V N V G T G V D H S  
271 I S E I A D M V A T A V G Y I G E T R W D P T K P D G T P R  
301 K L L D V S A L R E L G W R P R I A L K D G I D A T V S W Y  
331 R T N A D A V R R

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## Seq. ID No.15

1 gtgcgatggc acaccatgga tcgacacgcc gatgttgcc tgggtggggc gagtaagttg  
 61 acgactacac ctgggcctct ggaccgcgca acgcccggtg atatcgccgg tcatcggggg  
 121 ctgggtcggt cagcgctcgt acgtagattt gaggccgagg gggttcaccaa tctcattgtg  
 181 cgatcacgcg atgagattga tctgacggac cgagccgcaa cgtttgattt tgtgtctgag  
 241 acaagaccac aggtgatcat cgatgcggcc gcacgggtcg gcggcatcat gggaataaac  
 301 acctatcccg cggacttctt gtccgaaaac ctccgaatcc agaccaattt gtcgacgca  
 361 gctgtcgccg tgcgtgtgcc gcggctcctt ttctcgggtt cgtcatgcat ctaccggaag  
 421 tacgctccgc aacctatcca cgagagtgtt ttattgactg gccctttgga gccaccaaac  
 481 gacgcgtatg cgatcgccaa gatcgccggt atcctgcaag ttcaggcggg tagggcgcga  
 541 tatgggctgg cgtggatctc tgcgatgccg actaacctct acggaccctg cgacaacttc  
 601 tccccgtccg gggtcgatct ctgtccggcg ctcatccgtc gatatgagga agccaaagct  
 661 ggtggtgcag aagaggtgac gaattggggg accgggtactc cgccggcgca acttctgcat  
 721 gtcgacgacg tggcgagcgc atgcctgttc cttttggaac atttcgatgg tccgaaccac  
 781 gtcaacgtgg gcaccggcgt cgatcacagc attagcgaga tcgcagacat ggtcgctacg  
 841 gcgggtgggt acatcggcga aacacgttgg gatccaacta aacccgatgg aaccccgcg  
 901 aaactattgg acgtctccgc gctacgcgag ttgggttggc gcccggaat cgcactgaaa  
 961 gacggcatcg atgcaacggt gtcgtgttac cgcacaaatg ccgatgccgt gaggaggtaa

## Seq. ID No.16

1 V R W H T M D R H A D V A W L G R S K L T T T P G P L D R A  
 31 T P V Y I A G H R G L V G S A L V R R F E A E G F T N L I V  
 61 R S R D E I D L T D R A A T F D F V S E T R P Q V I I D A A  
 91 A R V G G I M A N N T Y P A D F L S B N L R I Q T N L L D A  
 121 A V A V R V P R L L F L G S S C I Y P K Y A P Q P I H E S A  
 151 L L T G P L E P T N D A Y A I A K I A G I L Q V Q A V R R Q  
 181 Y G L A W I S A M P T N L Y G P G D N F S P S G S H L L P A  
 211 L I R R Y E E A K A G G A E E V T N W G T G T P R R E L L H  
 241 V D D L A S A C L F L L E H F D G P N H V N V G T G V D H S  
 271 I S E I A D M V A T A V G Y I G E T R W D P T K P D G T P R  
 301 K L L D V S A L R E L G W R P R I A L K D G I D A T V S W Y  
 331 R T N A D A V R R

## Seq. ID No.17

1 atggattttt tgcgcaacgc cggcttgatg gctcgtaacg ttagtaccga gatgctgcgc  
 61 cacttcgaac gaaagcgctt attagtaaac caattcaaag catacggagt caacgttgtt  
 121 attgatgtcg gtgctaactc cggccagttc ggtagcgctt tgcgtcgtgc aggtattcaag  
 181 agccgtatcg ttctctttga acctctttcg gggccatttg cgcaactaac gcgcaagtcg  
 241 gcacgcgacg cactatggga gtgtcaccag tatgccctag gcgacgccga tgagacgatt  
 301 accatcaatg tggcaggcaa tgcgggggca agtagttccg tgcgtccgat gcttaaaagt  
 361 catcaagatg ctttctctcc cgcgaattat attggcaccg aagacgttgc aatacaccgc  
 421 cttgattcgg ttgcatcaga atttctgaac cctaccgatg ttactttcct gaagatcgac  
 481 gtacagggtt tcgagaagca ggttatcacg ggcagtaagt caacgcttaa cgaaagctgc  
 541 gtcggcatgc aactcgaact ttcttttatt ccgttgtagc aaggtgacat gctgattcat  
 601 gaagcgcttg aacttgtcta ttccctaggt ttcagactga cgggtttgtt gcccggtttt  
 661 acggatccgc gcaatggtcg aatgcttcaa gctgacggca ttttcttccg tggggacgat  
 721 tga

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## Seq. ID No.18

1 M D F L R N A G L M A R N V S T E M L R H F E R K R L L V N  
 31 Q F K A Y G V N V V I D V G A N S G Q F G S A L R R A G F K  
 61 S R I V S F E P L S G P P A Q L T R K S A S D P L W E C H Q  
 5 91 Y A L G D A D E T I T I N V A G N A G A S S S V L P M L K S  
 121 H Q D A F P P A N Y I G T E D V A I H R L D S V A S E F L N  
 151 P T D V T F L K I D V Q G F E K Q V I T G S K S T L N E S C  
 181 V G M Q L E L S F I P L Y E G D M L I H E A L E L V Y S L G  
 211 F R L T G L L P G F T D P R N G R M L Q A D G I F F R G D D

## 10 Seq. ID No.19

1 atggattttt tgcgcaacgc cggcttgatg gctcgtaacg ttagcaccga gatgctgcgc  
 61 cacttcgaac gaaagcgccct attagtaaac caattcaaag catacggagt caacgttggt  
 121 attgatgtcg gtgctaactc cggccagtcc ggtagcgctt tgcgtcgtgc aggattcaag  
 15 181 agcggatcgc tttcctttga acctctttcg gggccatttg cgcaactaac gcgcgagtcg  
 241 gcatcggatc cactatggga gtgtcaccag tatgccctag gcgacgccga tgagacgatt  
 301 accatcaatg tggcaggcaa tgcgggggca agtagttccg tgcgtccgat gcttaaaagt  
 361 catcaagatg cctttcctcc cgcgaattat attggcaccg aagacgttgc aatacaccgc  
 421 cttgattcgg ttgcatcaga atttctgaac cctaccgatg ttactttcct gaagatcgac  
 481 gtacaggggt tcgagaagca ggttatcgcg ggcagtaagt caacgcttaa cgaaagctgc  
 20 541 gtcggcatgc aactcgaact ttcttttatt ccgttgtagc aaggtagacat gctgattcat  
 601 gaagcgcttg aacttgctta ttccctaggt ttcagactga cgggtttggt gcccgattt  
 661 acggatccgc gcaatggctg aatgcttcaa gctgacggca ttttcttccg tggggacgat  
 721 tga

## Seq. ID No.20

25 1 M D F L R N A G L M A R N V S T E M L R H F E R K R L L V N  
 31 Q F K A Y G V N V V I D V G A N S G Q F G S A L R R A G F K  
 61 S R I V S F E P L S G P P A Q L T R E S A S D P L W E C H Q  
 91 Y A L G D A D E T I T I N V A G N A G A S S S V L P M L K S  
 121 H Q D A F P P A N Y I G T E D V A I H R L D S V A S E F L N  
 30 151 P T D V T F L K I D V Q G F E K Q V I A G S K S T L N E S C  
 181 V G M Q L E L S F I P L Y E G D M L I H E A L E L V Y S L G  
 211 F R L T G L L P G F T D P R N G R M L Q A D G I F F R G D D

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## Seq. ID No.21

1 atgactgcgc cagtgttctc gataattatc cctaccttca atgcagcggg gacgctgcaa  
61 gcctgcctcg gaagcatcgt cgggcagacc taccgggaag tggaaagtgg ccttgtcgac  
121 ggcgggttcga ccgacgcggc cctcgacatc gcgaacagtt tccgcccggg actcggctcg  
5 181 cgactggtcg ttcacagcgg gcccgatgat ggcccctacg acgccatgaa ccgcggcgctc  
241 ggcgtggcca caggcgaatg ggtacttttt ttaggcgcgg acgacaccct ctacgaacca  
301 accacgttgg cccaggtagc cgcttttctc ggcgaccatg cggcaagcca tcttgtctat  
361 ggcgatgttg tgatgcgttc gacgaaaagc cggcatgccg gacctttcga cctcgaccgc  
421 ctcttatttg agacgaattt gtgccaccaa tcgatctttt accgccgtga gcttttcgac  
10 481 ggcacgcggc cttacaacct gcgctaccga gtctgggcgg actgggactt caatattcgc  
541 tgcttctcca acccggcgct gattaccgcg tacatggacg tcgtgatttc cgaatacaac  
601 gacatgaccg gcttcagcat gaggcagggg actgataaag agttcagaaa acggctgcca  
661 atgtacttct gggttgcagg gtgggagact tgcaggcgca tgctggcggt tttgaaagac  
721 aaggagaatc gccgtctggc cttgcgtacg cggttgataa gggttaaggc cgtctccaaa  
15 781 gaacgaagcg cagaaccgta g

## Seq. ID No.22

1 M T A P V F S I I I P T F N A A V T L Q A C L G S I V G Q T  
31 Y R E V E V V L V D G G S T D R T L D I A N S F R P E L G S  
61 R L V V H S G P D D G P Y D A M N R G V G V A T G E W V L F  
20 91 L G A D D T L Y E P T T L A Q V A A F L G D H A A S H L V Y  
121 G D V V M R S T K S R H A G P P D L D R L L F E T N L C H Q  
151 S I F Y R R E L F D G I G P Y N L R Y R V W A D W D F N I R  
181 C F S N P A L I T R Y M D V V I S E Y N D M T G F S M R Q G  
211 T D K E F R K R L P M Y F W V A G W E T C R R M L A P L K D  
25 241 K E N R R L A L R T R L I R V K A V S K E R S A E P

## Seq. ID No.23

1 atgactgcgc cagtgttctc gataattatc cctaccttca atgcagcggg gacgctgcaa  
61 gcctgcctcg gaagcatcgt cgggcagacc taccgggaag tggaaagtgg ccttgtcgac  
121 ggcgggttcga ccgacgcggc cctcgacatc gcgaacagtt tccgcccggg actcggctcg  
30 181 cgactggtcg ttcacagcgg gcccgatgat ggcccctacg acgccatgaa ccgcggcgctc  
241 ggcgtagcca caggcgaatg ggtacttttt ttaggcgcgg acgacaccct ctacgaacca  
301 accacgttgg cccaggtagc cgcttttctc ggcgaccatg cggcaagcca tcttgtctat  
361 ggcgatgttg tgatgcgttc gacgaaaagc cggcatgccg gacctttcga cctcgaccgc  
421 ctcttatttg agacgaattt gtgccaccaa tcgatctttt accgccgtga gcttttcgac  
35 481 ggcacgcggc cttacaacct gcgctaccga gtctgggcgg actgggactt caatattcgc  
541 tgcttctcca acccggcgct gattaccgcg tacatggacg tcgtgatttc cgaatacaac  
601 gacatgaccg gcttcagcat gaggcagggg actgataaag agttcagaaa acggctgcca  
661 atgtacttct gggttgcagg gtgggagact tgcaggcgca tgctggcggt tttgaaagac  
721 aaggagaatc gccgtctggc cttgcgtacg cggttgataa gggttaaggc cgtctccaaa  
40 781 gaacgaagcg cagaaccgta g

## Seq. ID No.24

1 M T A P V F S I I I P T F N A A V T L Q A C L G S I V G Q T  
 31 Y R E V E V V L V D G G S T D R T L D I A N S F R P E L G S  
 61 R L V V H S G P D D G P Y D A M N R G V G V A T G E W V L F  
 5 91 L G A D D T L Y E P T T L A Q V A A F L G D H A A S H L V Y  
 121 G D V V M R S T K S R H A G P F D L D R L L P E T N L C H Q  
 151 S I F Y R R E L F D G I G P Y N L R Y R V W A D W D F N I R  
 181 C P S N P A L I T R Y M D V V I S E Y N D M T G F S M R Q G  
 211 T D K E F R K R L P M Y F W V A G W E T C R R M L A F L K D  
 10 241 K E N R R L A L R T R L I R V K A V S K E R S A E P

## Seq. ID No.25

1 gtggccagca gaagtccecca ctccgctgcg ggtggttggc taattcttgg cggctccctt  
 61 ctgtggtcg gcgtggcgca tccggtaggc ctccgctggc gtgacgacga tgctggcggtg  
 121 gtgcagcagc cgatcgagga tgctggcggc ggtggtgtgc tcgggcagga atcgcececca  
 15 181 ttgttcgaag ggccaatgag aggcgatggc caggagcggc cgctcgtagc cggcagccac  
 241 gagccggaac aacagttgag tcccggtgtc gtcgagcggc gcgaagccga tctcgctcaa  
 301 gatgaccaga tccgcgcgga gcagggtgtc gatgatcttg ccgacggtgt tgctggccag  
 361 gccgcggtg aggacctcga tcagggtcggc ggcggtgaag tagcggactt tgaatccggc  
 421 gtggacggca gcgtgcccgc agccgatgag cagggtgactt ttgcccgtac cagggtgggccc  
 20 481 aatgaccgcc aggttctgtt gtgcccgaat ccattccagg ctgcacaggt agtcgaacgt  
 541 ggctgcggtg atcgacgacg cggtgacgtc gaaccgcgtc agggctcttg tgaccgggaa  
 601 ggctgcggtc ttgagacggt tggcggtgtt ggaggcatcg cgggcagcga tctcgccctc  
 661 aaccaacgtc cgcaggatct cctccggtgt ccagcggtgc gtcttgggga cttgcaaacac  
 721 ctcgggcgcg ttgcggcgca ccgtggccag cttcaaccgc cgcagcgccg cgtcaaggtc  
 25 781 agcagccagc ggtgccgccc aggacggtgc caccggcttg gcagcggtgg tcatgaggcc  
 841 gtcccgtcgg tgggtgtgat cttgtag

## Seq. ID No.26

1 V A S R S P H S A A G G W L I L G G S L L V V G V A H P V G  
 31 L A G G D D D A G V V Q Q P I E D A G G G G V L G Q E S P P  
 61 L F E G P M R G D G Q G A A L V A G S H E P E Q Q L S P G V  
 91 V E R G E A D L V Q D D Q I R A E Q G V D D L A D G V V G Q  
 121 A A V E D L D Q V G G G E V A D F E S G V D G S V P A A D E  
 151 Q V T F A R T R W A N D R Q V L L C P N P F Q A R Q V V E R  
 181 G C G D R R S G D V E P V E G L G D R E G C G L E T V G G V  
 211 G G I A G S D L G L N Q R P Q D L L R C P A L R L G D L Q H  
 241 L G G V A A H R G Q L O P P O R R V K V S S Q R C R R G R C  
 35 271 H R L G S G G H E A V P S V V L I L

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## Seq. ID No.27

1 atgggctgcc tcaaagggtgg tgtcgtcgcc aatggtgttg ttccaacacc ggattatgtg  
61 cgattcgcgt cccactatgg ctctgttccg gacttctgcc acggtgcgga tccgcaatcg  
121 aagggcatcg tggagaacct ctgtggctac gctcaggacg accttgccgt gccgctgctg  
5 181 accgaagctg cgttagccgg tgagcaggtc gacctacgtg ccttcaacgc ccaggcgcaa  
241 ctatgggtcg ccgaggtcaa tgccacggtc cactcggaga tctgcgccgt gcccaacgat  
301 cgcttggttg acgagcgcac cgtcttgagg gagctgccct cgctgcggcc gacgatcggc  
361 tcgggggtcgg tgcgcccgtaa ggtcgcaggc ctctcgtgca tccgttacgg ctacagctcgt  
421 tactcgggtg ctcagcggct cgtcgggtgcc accgtggcgg tgggtggtcga tcatggcgcc  
10 481 ctgatcctgt tggaaacctgc gaccgggtgtg atcgtggccg agcacgagct cgtcagccca  
541 ggtgaggtgt ccatcctcga tgaacactac gacggaccca gaccgcacc ctcgcgtggt  
601 cctcgcgccg aaacccaagc agagaacaga ttctgcgcat tgggaaccga agcgcagcag  
661 ttcctcgtcg gtgctgctgc gatcggcaac acccgactga aatccgaact cgacattctg  
721 ctcggccttg gcgcgcacca cggcgaacag gctttgattg acgcgctcgc ccgggcggtt  
15 781 gcgtttcgcc gggtccgcgc tgcgcacgtg cgtcgcgacc tggccgccgg cgccggcacc  
841 ccacaacccc gccccgcggg cgacgcactc gtgctcgatc tgcccaccgt cgagaccgcg  
901 tcgttggagg cctacaagat caacaccacc gacgggacgg cctcatgacc accgctgcc  
961 agccggtggc accgtcctcg gcggcaccgc tggctgctga ccttgacgcg gcgctgcggc  
1021 ggttgaagct ggccacggtg cgccgcaacg ccgcccagggt gttgcaagtc gccaaagcgc  
20 1081 aacgctggac accggaggag atcctgcgga cgttggttga ggccgagatc gctgcccgcg  
1141 atgcctcaa caccgccaac cgtctcaagg ccgcagcctt cccggtcacc aagaccctcg  
1201 acgggttcga cgtcaccgga tcgtcgatca ccgcagccac gttcgactac ctgtcgagcc  
1261 tggaaatgat tcggggacaa cagaacctgg cggtcattgg cccacctggt acggggcaaaa  
1321 gtcacctgct catcggtgc gggcacgctg ccgtccacgc cggattcaaa gtcgctact  
25 1381 tcaccgcgcg cgacctgatc gaggtcctct accgcggcct ggccgacaac accgtcggca  
1441 agatcatcga caccctgctc cgcgcggatc tggtcattct ggacgagatc ggcttcgccc  
1501 cgctcgacga caccgggact caactgttgt tccggctcgt ggctgccggc tacgagcgcc  
1561 gtcacctggc catcgctcg cattggccct tcgaacaatg ggggcgattc ctgcccgagc  
1621 acaccaccgc cgccagcatc ctcgatcggc tgctgcacca cgccagcatc gtcgtcacct  
30 1681 ccggcgagtc ctaccggatg cgccacgccc accacaagaa gggagccgcc aagaattag

## Seq. ID No.28

1 M G C L K G G V V A N V V V P T P D Y V R P A S H Y G F V P  
31 D F C H G A D P Q S K G I V E N L C G Y A Q D D L A V P L L  
61 T E A A L A G E Q V D L R A L N A Q A Q L W C A E V N A T V  
91 H S E I C A V P N D R L V D E R T V L R E L P S L R P T I G  
121 S G S V R R K V D G L S C I R Y G S A R Y S V P Q R L V G A  
151 T V A V V V D H G A L I L L E P A T G V I V A E H E L V S P  
181 G E V S I L D E H Y D G P R P A P S R G P R P K T Q A B K R  
211 F C A L G T E A Q Q F L V G A A A I G N T R L X S E L D I L  
40 241 L G L G A A H G E Q A L I D A L R R A V A F R R F R A A D V  
271 R S I L A A G A G T P Q P R P A G D A L V L D L P T V E T R  
301 S L E A Y K I N T T D G T A S

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## Seq. ID No.29

1 MTTAAKPVAPSSAAPLAADLDAALRRRLKLA  
 31 TVRRNAAEVLQVAKTORWTP EEILRTLVEA  
 61 EIAARDASNTANRLKAAAPVTKTLDGFDV  
 5 91 TGSSITAAATFDYLS SLEWIRAQQNLAVIGP  
 121 PGTGKSHLLIGCGHAAVHAGFKVRYFTAAD  
 151 LIEVLYRGLADNTVGKIIDTLLRADLVILD  
 181 BIGFAPLDDTGTQLLFRLVAAGYERRSLAI  
 211 ASHWPF EQWGRFLPBHTTAASILDRLLHHA  
 10 241 SIVVTSGESYRNRHADHKKGAANK

## Seq. ID No.30

1 gtgacgtctg ctccgaccgt ctcggtgata acgatctcgt tcaacgacct cgacgggttg  
 61 cagegcacgg tgaaaagtgt gcgggcgcaa cgctaccggg gacgcatcga gcacatcgta  
 121 atcgacgggtg gcagcggcga cgacgtgggtg gcatacctgt cggggtgtga accaggcttc  
 15 181 gcgtattggc agtccgagcc cgacggcggg cggtacgacg cgatgaacca gggcatcgcg  
 241 cacgcatcgg gtgatctgtt gtggttcttg cactccgccg atcgtttttc cgggcccgac  
 301 gtggtagccc aggcctgtga ggcgctatcc ggcaaggagc cgggtgtccga attgtggggc  
 361 ttcgggatgg atcgctctcgt cgggctcgat cgggtgcgcg gcccgatacc ttccagcctg  
 421 cgcaaattcc tggccggcaa gcagggtgtt ccgcatcaag catcgttctt cggatcatcg  
 20 481 ctggtggcca agatcgggtg ctacgacctt gatttcggga tcgccgccga ccaggaattc  
 541 atattcggg ccgcgctggt atgcgagccg gtcacgattc ggtgtgtgct gtgcgagttc  
 601 gacaccacgg gcgtcggctc gcaccgggaa ccaagcgcg tcttcggtga tctgcgccgc  
 661 atgggcgacc ttcacgcgcg ctacccttc gggggaaggc gaatatcaca tgcctaccta  
 721 cgcggccggg agttctacgc ctacaacagt cgattctggg aaaacgtctt cgcgcaatg  
 25 781 tcgaatatg

## Seq. ID No.31

1 MTSAPT VSVITISFNDLDGLQRTVKSVRAQ  
 31 RYRGRIEHIVIDGGS GDDVVAYLSGCEPGF  
 61 AYWQSEPDGGRYDAMNOGIAHASGDLLWFL  
 30 91 HSADRFSGPDVVAQAVEALS GKGPVSELWG  
 121 FGMDRLVGLDRVRGP I PFS LRKFLAGKQVV  
 151 PHQASFFGSSLVAKIGGYDLDFGIAADQEF  
 181 ILRAALVCEPVTIRCVLCBFDTTGVGSHRE  
 211 PSAVFGLRRMGDLHRRYPFGGRRISHAYL  
 35 241 RGREFYAYNSRFWENVFTRMSK

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## Seq. ID No.32

1 gtgaagcgag cgctcatcac cggaaacacc ggccaggacg gctcgtatct cgccgaactg  
 61 ctgctggcca aggggtatga gggtcacggg ctcatccggc gcgcttcgac gttcaacacc  
 121 tcgcggatcg atcacctcta cgtcgacccg caccaaccgg gcgcgcggct gtttctgcac  
 5 181 tatggtgacc tgatcgacgg aaccgggttg gtgaccctgc tgagcaccat cgaaccggac  
 241 gaggtgtaca acctggcggc gcagtcacac gtgcgggtga gtttcgacga acccgtgcac  
 301 accggtgaca ccaccggcat gggatccatg cgactgctgg aagccgttcg gctctctcgg  
 361 gtgcactgcc gcttctatca ggcgtcctcg tcggagatgt tcggcgccct gccgccaccg  
 421 cagaacgagc tgacgcggtt ctacccgcgg tcaccgtatg gcgccgcca ggtctattcg  
 10 481 tactggggcga ccgcaatta tcgcgaagcg tacggattgt tcgccgttaa eggcatcttg  
 541 ttcaatcacg aatcacccgg gcgcgggtgag acgttcgtga ccgaaagat caccagggcc  
 601 gtggcacgca tcaaggccgg tatccagtcg gaggtctata tgggcaatct ggatgcggtc  
 661 cgcgactggg ggtacgcgcc cgaatacgtc gaaggcatgt ggcggatgct gcagaccgac  
 721 gagcccgacg acttcgtttt ggcgaccggg cgcggtttca ccgtgcgtga gttcgcgcgg  
 15 781 gccgcgttcg agcatgccgg tttggactgg cagcagtcag tgaaattcga ccaacgctat  
 841 ctgcggccca ccgaggtgga ttcgctgacg ggcgacgcga ccaaggctgc cgaattgctg  
 901 ggctggaggg cttcggtgca cactgacgag ttggtcggga tcatggtcga cgcggacatg  
 961 gcggcgctgg agtgcaagg caagccgtgg atcgacaagc cgatgatcgc cggccggaca  
 1021 tga

## 20 Seq. ID No.33

1 M K R A L I T G I T G Q D G S Y L A E L L L A K G Y E V H G  
 31 L I R R A S T F N T S R I D H L Y V D P H Q P G A R L F L H  
 61 Y G D L I D G T R L V T L L S T I E P D E V Y N L A A Q S H  
 91 V R V S F D E P V H T G D T T G N G S M R L L E A V R L S R  
 121 V H C R F Y Q A S S S E M F G A S P P P Q N E L T P F Y P R  
 25 151 S P Y G A A K V Y S Y W A T R N Y R E A Y G L F A V N G I L  
 181 F N H E S P R R G E T F V T R K I T R A V A R I K A G I Q S  
 211 E V Y M G N L D A V R D W G Y A P E Y V E G M W R M L Q T D  
 241 E P D D F V L A T G R G F T V R E F A R A A F E H A G L D W  
 30 271 Q Q Y V K F D Q R Y L R P T E V D S L I G D A T K A A E L L  
 301 G W R A S V H T D E L A R I M V D A D M A A L E C E G K P W  
 331 I D K P M I A G R T

## Seq. ID No.34

1 atgaggctgg ccgctcgcgc tcggaacatc ttgcgtcgca acggcatcga ggtgtcgcgc  
 35 61 tactttgccg aactggactg ggaacgcaat ttcttgccg aactgcaatc gcatcgggtc  
 121 agtgccgtgc tcgatgtcgg ggccaattcg ggcagtcag ccagggggtct gcgcggcgcg  
 181 ggcttcgcgg gccgcatcgt ctggttcgag ccgctgcccc ggccctttgc cgtcttcgag  
 241 cgcagcgccct ccacggaccc gtgtgtggaa tgccggcgct gtgcgctggg cgatgtcgat  
 301 ggaaccatct cgatcaacgt cgcgggcaac gagggcgcca gcagttccgt cttgccgatg  
 40 361 ttgaaacgac atcaggacgc cttccacca gccaaactacg tgggcgcccc acgggtgccg  
 421 atacatcgac tcgattccgt ggctgcagac gttctgcggc ccaacgatat tgcgttcttg  
 481 aagatcgacg ttcaaggatt cgagaagcag gtgatcgcg gtggcgattc aacggtgac  
 541 gaccgatgcg tcggcatgca gctcgagctg tctttccagc cgttgtagca ggggtggcatg  
 601 ctcatccgcg aggcgctcga tctcgtggat tcgttgggct ttacgctctc gggattgcaa  
 45 661 cccggtttca ccgaccccc caacggtcga atgctgcagg ccgatggcat cttcttccgg  
 721 ggcagcgatt ga

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## Seq. ID No.35

1 M R L A R R A R N I L R R N G I E V S R Y F A E L D W E R N  
 31 F L R Q L Q S H R V S A V L D V G A N S G Q Y A R G L R G A  
 61 G F A G R I V S F E P L P G P F A V L Q R S A S T D P L W E  
 5 91 C R R C A L G D V D G T I S I N V A G N E G A S S S V L P M  
 121 L K R H Q D A F P P A N Y V G A O R V P I H R L D S V A A D  
 151 V L R P N D I A F L K I D V Q G F E K Q V I A G G D S T V H  
 181 D R C V G M Q L E L S F Q P L Y E G G M L I R E A L D L V D  
 211 S L G F T L S G L Q P G F T D P R N G R M L Q A D G I F F R  
 10 241 G S D

## Seq. ID No.36

1 gtgaaatcgt tgaactcgc tcgtttcacc gcgcgtagcg ccgccttcga ggtttcgcgc  
 61 cgctattctg agcgagacct gaagcaccag tttgtgaagc aactcaaatc gcgtcgggta  
 121 gatgtcggtt tcgatgtcgg cgccaactca ggacaatacg ccgccggcct ccgccgagca  
 15 181 gcatataagg gccgcattgt ctcttcgaa ccgctatccg gaccgtttac gatcttgaa  
 241 agcaaacgct caacggatcc actttgggat tgcggcagc atgcgttggg cgattctgat  
 301 ggaacgggta cgatcaatat cgaggaaac gccggtcaga gcagtccgt cttgcccatg  
 361 ctgaaaagtc atcagaacgc tttcccccg gcaaaactatg tcggtaccca agaggcgtcc  
 421 atacatcgac ttgattccgt ggcgccagaa tttctaggca tgaacgggtg cgcttttctc  
 20 481 aaggctcgacg ttcaaggctt tgaagagcag gtgctcgccg ggggcaaatc aaccatagat  
 541 gaccattgag tcggcatgca actcgaactg tccttcctgc cgttgtagc aggtggcatg  
 601 ctcatctctg aagccctcga tctcgtgtat tccttgggct tcacgttgac gggattgctg  
 661 ccttgtttca ttgatgcaaa taatggtcga atgttgacgg ccgacggcat cttttccgc  
 721 gaggacgatt ga

## 25 Seq. ID No.37

1 M K S L K L A R F I A R S A A F E V S R R Y S E R D L K H Q  
 31 F V K Q L K S R R V D V V F D F T V G A N S G Q Y A A G L R  
 61 R A A Y K G R I V S F E P L S G P F T I L E S K A S T D P L  
 91 W D C R Q H A L G D S D G T V T I N I A G N A G Q S S S V L  
 121 P M L K S H Q N A F P P A N Y V G T Q E A S I H R L D S V A  
 151 P E F L G M N G V A F L K V D V Q G P E K Q V L A G G K S T  
 181 I D D H C V G M Q L E L S F L P L Y E G G M L I P E A L D L  
 211 V Y S L G F T L T G L L P C F I D A N N G R M L Q A D G I F  
 241 F R E D D

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## Seq. ID No.38

1 atggtgcaga cgaaacgata cgccggcttg accgcagcta acacaaagaa agtcgccatg  
 61 gccgcaccaa tgttttcgat catcatcccc accttgaacg tggctgcggt attgacctgcc  
 121 tgcctcgaca gcatcgcccc tcagacctgc ggtgacttcg agctggtact ggtcgacggc  
 5 181 ggctcgacgg acgaaaccct cgacatcgcc aacattttcg cccccaacct cggcgagcgg  
 241 ttgatcatc atcgcgacac cgaccagggc gtctacgacg ccatagaaccg cggcggtggac  
 301 ctggccaccg gaacgtggtt gctctttctg ggcgcgagc acagcctgta cgaggctgac  
 361 accctggcgc ggggtggcgc cttcattggc gaacacgagc ccagcgatct ggtatatggc  
 421 gacgtgatca tgcgctcaac caatttccgc tggggtggcg ccttcgacct cgaccgtctg  
 10 481 ttgttcaagc gcaacatctg ccatacaggcg atcttctacc gccgcggact cttcggcacc  
 541 atcgggtccct acaacctccg ctaccgggtc ctggcggact gggacttcaa tattcgctgc  
 601 ttttccaacc cagcgctcgt caccgcgtac atgcacgtgg tcgttgcaag ctacaacgaa  
 661 ttggcggggc tcagcaatac gatcgtcgac aaggagtttt tgaagcggct gccgatgtcc  
 721 acgagactcg gcataaggct ggtcatagtt ctggtgcgca ggtggccaaa ggtgatcagc  
 15 781 agggccatgg taatgcgcac cgtcatttct tggcggcgcc gacgttag

## Seq. ID No.39

1 M V Q T K R Y A G L T A A N T K K V A M A A P M F S I I I P  
 31 T L N V A A V L P A C L D S I A R Q T C G D F E L V L V D G  
 61 G S T D E T L D I A N I F A P N L G E R L I I H R D T D O G  
 20 91 V Y D A M N R G V D L A T G T W L L F L G A D D S L Y E A D  
 121 T L A R V A A F I G E H E P S D L V Y G D V I M R S T N F R  
 151 W G G A F D L D R L L F K R N I C H Q A I F Y R R G L F G T  
 181 I G P Y N L R Y R V L A D W D F N I R C F S N P A L V T R Y  
 211 M H V V V A S Y N E F G G L S N T I V D K E F L K R L P M S  
 25 241 T R L G I R L V I V L V R R W P K V I S R A M V M R T V I S  
 271 W R R R R

## Seq 40:

GATGCCGTGAGGAGGTAAAGCTGC

## Seq 41:

30 GATACGGCTCTTGAATCCTGCACG

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CLAIMS

1. A polypeptide in substantially isolated form which comprises any one of the sequences selected from the group consisting of Seq.ID.No: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 35, 37 and 39, or a polypeptide substantially homologous thereto.

2. A polypeptide in substantially isolated form which comprises any one of the sequences selected from the group consisting of Seq.ID.No: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 35, 37 and 39.

3. A polypeptide which comprises a fragment of a polypeptide defined in claim 1 or 2, said fragment comprising at least 12 amino acids and an epitope.

4. A polynucleotide in substantially isolated form which encodes a polypeptide according to any one of claims 1 to 3.

5. A polynucleotide in substantially isolated form which is capable of selectively hybridizing to SEQ ID NO: 3 or 4 or a fragment thereof.

6. A polynucleotide fragment according to claim 5 which comprises a sequence selected from the group consisting of Seq.ID.No: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27, or a polynucleotide at least 90% homologous thereto.

7. A polynucleotide in substantially isolated form comprising a sequence selected from the group consisting of Seq.ID.No: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27.

8. A polynucleotide in substantially isolated form consisting essentially of a sequence selected from the group Seq ID Nos. 30, 32, 34, 36 and 38, or a polynucleotide at least 90% homologous thereto.

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9. A polynucleotide in substantially isolated form consisting essentially of a sequence selected from the group Seq ID Nos. 30, 32, 34, 36 and 38.

10. A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a polynucleotide as defined in any one of claims 4 to 8, optionally carrying a revealing label.

11. A recombinant vector carrying a polynucleotide as defined in any one of claims 4 to 8.

12. An antibody capable of binding a polypeptide or fragment thereof as defined in any one of claims 1 to 3.

13. A test kit for detecting the presence or absence of a pathogenic mycobacterium in a sample which comprises a polynucleotide according to any one of claims 4 to 10, a polypeptide according to any one of claims 1 to 3, or an antibody according to claim 12.

14. A method of detecting the presence or absence of antibodies in an animal or human, against a pathogenic mycobacteria in a sample which comprises:

- (a) providing a polypeptide according to any one of claims 1 to 3 comprising an epitope;
- (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and
- (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

15. A method of detecting the presence or absence of a polypeptide according to any one of claims 1 to 3 in a biological sample which method which comprises:

- (a) providing an antibody according to claim 11;
- (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and

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- (c) determining whether antibody-antigen complex comprising said antibody is formed.

16. A method of detecting the presence or absence of cell mediated immune reactivity in an animal or human, to a polypeptide according to claims 1 to 3 which method comprises

- (a) providing a polypeptide according to any one of claims 1 to 3 comprising an epitope;
- (b) incubating a cell sample with said polypeptide under conditions which allow for a cellular immune response such as release of cytokines or other mediator or reaction to occur; and
- (c) detecting the presence of said cytokine or mediator or cellular response in the incubate.

17. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 3 in a suitable carrier or diluent.

18. A composition according to claim 17 for use in the treatment or prevention of diseases caused by mycobacteria.

19. A method of treating or preventing mycobacterial disease in an animal or human caused by mycobacteria which express a polypeptide according to claims 1 to 3, which method comprises vaccinating or treating an animal or human with an effective amount of said polypeptide.

20. A method of treating or preventing mycobacterial diseases in animals or humans caused by mycobacteria containing the polynucleotide of SEQ ID NO: 3 or 4, which method comprises vaccinating or treating an animal or human with an effective amount of a polynucleotide according to claims 4 to 9, or a vector according to claim 11.

21. A method according to claims 19 or 20 for increasing the in vivo susceptibility of mycobacteria to antimicrobial drugs.

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22. A vaccine comprising a normally pathogenic mycobacteria, which pathogenicity is mediated in all or in part by the presence of the expression of a polypeptide as defined in any one of claims 1 to 3, which mycobacteria harbours an attenuating mutation in any one of said genes.

23. A vaccine according to claim 22 wherein the mycobacteria is selected from *Mavs*, *Mptb* and *Mtb*.

Figure 1 a)

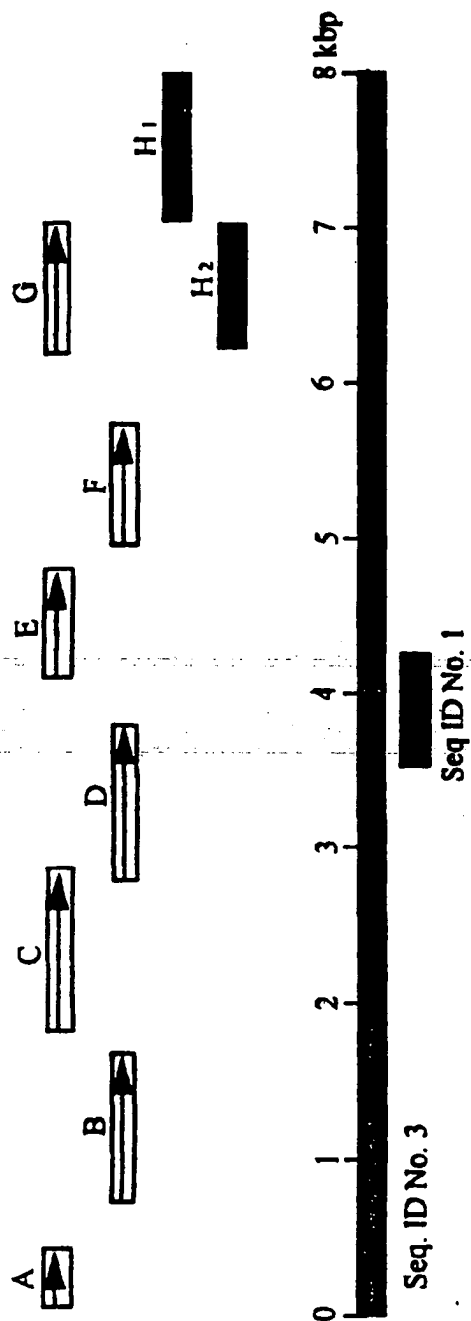


Figure 1 b)

